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(71) Applicant: BASF AKTIENGESELLSCHAFT [DE/DE]; D-67056 Ludwigshafen (DE).

(72) Inventors: POMPEJIUS, Markus; Wenjenstrasse 21, D-67251 Freinsheim (DE). KRÖGER, Burkhard; Im Waldhof 1, D-67117 Limburgerhof (DE). SCHRÖDER, Hartwig; Goethestrasse 5, D-69226 Nussloch (DE). ZELDER, Oskar; Rossmarktstrasse 27, D-67346 Speyer (DE). HABERHAUER, Gregor; Moselstrasse 42, D-67117 Limburgerhof (DE).

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(54) Title: *CORYNEBACTERIUM GLUTAMICUM* GENES ENCODING PROTEINS INVOLVED IN CARBON METABOLISM AND ENERGY PRODUCTION

(57) Abstract: Isolated nucleic acid molecules, designated SMP nucleic acid molecules, which encode novel SMP proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing SMP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated SMP proteins, mutated SMP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of SMP genes in this organism.

CORYNEBACTERIUM GLUTAMICUM GENES ENCODING PROTEINS INVOLVED IN CARBON METABOLISM AND ENERGY PRODUCTION

Related Applications

This application claims priority to prior U.S. Provisional Patent Application Serial No. 60/141031, filed June 25, 1999, U.S. Provisional Patent Application Serial No. 60/143208, filed July 9, 1999, and U.S. Provisional Patent Application Serial No. 60/151572, filed August 31, 1999. This application also claims priority to prior German Patent Application No. 19931412.8, filed July 8, 1999, German Patent Application No. 19931413.6, filed July 8, 1999, German Patent Application No. 19931419.5, filed July 8, 1999, German Patent Application No. 19931420.9, filed July 8, 1999, German Patent Application No. 19931424.1, filed July 8, 1999, German Patent Application No. 19931428.4, filed July 8, 1999, German Patent Application No. 19931431.4, filed July 8, 1999, German Patent Application No. 19931433.0, filed July 8, 1999, German Patent Application No. 19931434.9, filed July 8, 1999, German Patent Application No. 19931510.8, filed July 8, 1999, German Patent Application No. 19931562.0, filed July 8, 1999, German Patent Application No. 19931634.1, filed July 8, 1999, German Patent Application No. 19932180.9, filed July 9, 1999, German Patent Application No. 19932227.9, filed July 9, 1999, German Patent Application No. 19932230.9, filed July 9, 1999, German Patent Application No. 19932924.9, filed July 14, 1999, German Patent Application No. 19932973.7, filed July 14, 1999, German Patent Application No. 19933005.0, filed July 14, 1999, German Patent Application No. 19940765.7, filed August 27, 1999, German Patent Application No. 19942076.9, filed September 3, 1999, German Patent Application No. 19942079.3, filed September 3, 1999, German Patent Application No. 19942086.6, filed September 3, 1999, German Patent Application No. 19942087.4, filed September 3, 1999, German Patent Application No. 19942088.2, filed September 3, 1999, German Patent Application No. 19942095.5, filed September 3, 1999, German Patent Application No. 19942123.4, filed September 3, 1999, and German Patent Application No. 19942125.0, filed September 3, 1999. The entire contents of all of the aforementioned application are hereby expressly incorporated herein by this reference.

Background of the Invention

Certain products and by-products of naturally-occurring metabolic processes in cells have utility in a wide array of industries, including the food, feed, cosmetics, and pharmaceutical industries. These molecules, collectively termed 'fine chemicals', include organic acids, both proteinogenic and non-proteinogenic amino acids, nucleotides and nucleosides, lipids and fatty acids, diols, carbohydrates, aromatic compounds, vitamins and cofactors, and enzymes. Their production is most conveniently performed through the large-scale culture of bacteria developed to produce and secrete large quantities of one or more desired molecules. One particularly useful organism for this purpose is *Corynebacterium glutamicum*, a gram positive, nonpathogenic bacterium. Through strain selection, a number of mutant strains have been developed which produce an array of desirable compounds. However, selection of strains improved for the production of a particular molecule is a time-consuming and difficult process.

Summary of the Invention

The invention provides novel bacterial nucleic acid molecules which have a variety of uses. These uses include the identification of microorganisms which can be used to produce fine chemicals, the modulation of fine chemical production in *C. glutamicum* or related bacteria, the typing or identification of *C. glutamicum* or related bacteria, as reference points for mapping the *C. glutamicum* genome, and as markers for transformation. These novel nucleic acid molecules encode proteins, referred to herein as sugar metabolism and oxidative phosphorylation (SMP) proteins.

C. glutamicum is a gram positive, aerobic bacterium which is commonly used in industry for the large-scale production of a variety of fine chemicals, and also for the degradation of hydrocarbons (such as in petroleum spills) and for the oxidation of terpenoids. The SMP nucleic acid molecules of the invention, therefore, can be used to identify microorganisms which can be used to produce fine chemicals, *e.g.*, by fermentation processes. Modulation of the expression of the SMP nucleic acids of the invention, or modification of the sequence of the SMP nucleic acid molecules of the invention, can be used to modulate the production of one or more fine chemicals from a

microorganism (*e.g.*, to improve the yield or production of one or more fine chemicals from a Corynebacterium or Brevibacterium species).

The SMP nucleic acids of the invention may also be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof, or to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is nonpathogenic, it is related to species pathogenic in humans, such as *Corynebacterium diphtheriae* (the causative agent of diphtheria); the detection of such organisms is of significant clinical relevance.

The SMP nucleic acid molecules of the invention may also serve as reference points for mapping of the *C. glutamicum* genome, or of genomes of related organisms. Similarly, these molecules, or variants or portions thereof, may serve as markers for genetically engineered Corynebacterium or Brevibacterium species.

The SMP proteins encoded by the novel nucleic acid molecules of the invention are capable of, for example, performing a function involved in the metabolism of carbon compounds such as sugars or in the generation of energy molecules by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. Given the availability of cloning vectors for use in *Corynebacterium glutamicum*, such as those disclosed in Sinskey *et al.*, U.S. Patent No. 4,649,119, and techniques for genetic manipulation of *C. glutamicum* and the related *Brevibacterium* species (*e.g.*, *lactofermentum*) (Yoshihama et al, *J. Bacteriol.* 162: 591-597 (1985); Katsumata *et al.*, *J. Bacteriol.* 159: 306-311 (1984); and Santamaria *et al.*, *J. Gen. Microbiol.* 130: 2237-2246 (1984)), the nucleic acid molecules of the invention may be utilized in the genetic engineering of this organism to make it a better or more efficient producer of one or more fine chemicals. This improved production or efficiency of production of a fine chemical may be due to a direct effect of manipulation of a gene of the invention, or it may be due to an indirect effect of such manipulation.

There are a number of mechanisms by which the alteration of an SMP protein of the invention may directly affect the yield, production, and/or efficiency of production of a fine chemical from a *C. glutamicum* strain incorporating such an altered protein. The degradation of high-energy carbon molecules such as sugars, and the conversion of compounds such as NADH and $FADH_2$ to compounds containing high energy phosphate bonds via oxidative phosphorylation results in a number of compounds which themselves may be desirable fine chemicals, such as pyruvate, ATP, NADH, and a number of intermediate sugar compounds. Further, the energy molecules (such as ATP) and the reducing equivalents (such as NADH or NADPH) produced by these metabolic pathways are utilized in the cell to drive reactions which would otherwise be energetically unfavorable. Such unfavorable reactions include many biosynthetic pathways for fine chemicals. By improving the ability of the cell to utilize a particular sugar (*e.g.*, by manipulating the genes encoding enzymes involved in the degradation and conversion of that sugar into energy for the cell), one may increase the amount of energy available to permit unfavorable, yet desired metabolic reactions (*e.g.*, the biosynthesis of a desired fine chemical) to occur.

The mutagenesis of one or more SMP genes of the invention may also result in SMP proteins having altered activities which indirectly impact the production of one or more desired fine chemicals from *C. glutamicum*. For example, by increasing the efficiency of utilization of one or more sugars (such that the conversion of the sugar to useful energy molecules is improved), or by increasing the efficiency of conversion of reducing equivalents to useful energy molecules (*e.g.*, by improving the efficiency of oxidative phosphorylation, or the activity of the ATP synthase), one can increase the amount of these high-energy compounds available to the cell to drive normally unfavorable metabolic processes. These processes include the construction of cell walls, transcription, translation, and the biosynthesis of compounds necessary for growth and division of the cells (*e.g.*, nucleotides, amino acids, vitamins, lipids, etc.) (Lengeler *et al.* (1999) Biology of Prokaryotes, Thieme Verlag: Stuttgart, p. 88-109; 913-918; 875-899). By improving the growth and multiplication of these engineered cells, it is possible to increase both the viability of the cells in large-scale culture, and also to improve their rate of division, such that a relatively larger number of cells can survive in fermentor culture. The yield, production, or efficiency of production may be increased, at least

due to the presence of a greater number of viable cells, each producing the desired fine chemical. Also, many of the degradation products produced during sugar metabolism are utilized by the cell as precursors or intermediates in the production of other desirable products, such as fine chemicals. So, by increasing the ability of the cell to metabolize sugars, the number of these degradation products available to the cell for other processes should also be increased.

The invention provides novel nucleic acid molecules which encode proteins, referred to herein as SMP proteins, which are capable of, for example, performing a function involved in the metabolism of carbon compounds such as sugars and the generation of energy molecules by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. Nucleic acid molecules encoding an SMP protein are referred to herein as SMP nucleic acid molecules. In a preferred embodiment, the SMP protein participates in the conversion of carbon molecules and degradation products thereof to energy which is utilized by the cell for metabolic processes. Examples of such proteins include those encoded by the genes set forth in Table 1.

Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (*e.g.*, cDNAs, DNAs, or RNAs) comprising a nucleotide sequence encoding an SMP protein or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection or amplification of SMP-encoding nucleic acid (*e.g.*, DNA or mRNA). In particularly preferred embodiments, the isolated nucleic acid molecule comprises one of the nucleotide sequences set forth as the odd-numbered SEQ ID NOs in the Sequence Listing (*e.g.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7....), or the coding region or a complement thereof of one of these nucleotide sequences. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes to or is at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence set forth as an odd-numbered SEQ ID NO in the Sequence Listing (*e.g.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7....), or a portion thereof. In other preferred embodiments, the isolated nucleic acid molecule encodes one of the amino acid sequences set forth as an even-numbered SEQ ID NO in the Sequence Listing (*e.g.*, SEQ ID NO:2, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:8....).. The preferred SMP proteins of the present invention also preferably possess at least one of the SMP activities described herein.

In another embodiment, the isolated nucleic acid molecule encodes a protein or portion thereof wherein the protein or portion thereof includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of the invention (*e.g.*, a sequence having an even-numbered SEQ ID NO: in the Sequence Listing), *e.g.*, sufficiently homologous to an amino acid sequence of the invention such that the protein or portion thereof maintains an SMP activity. Preferably, the protein or portion thereof encoded by the nucleic acid molecule maintains the ability to perform a function involved in the metabolism of carbon compounds such as sugars or the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. In one embodiment, the protein encoded by the nucleic acid molecule is at least about 50%, preferably at least about 60%, and more preferably at least about 70%, 80%, or 90% and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an amino acid sequence of the invention (*e.g.*, an entire amino acid sequence selected those having an even-numbered SEQ ID NO in the Sequence Listing). In another preferred embodiment, the protein is a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of the invention (encoded by an open reading frame shown in the corresponding odd-numbered SEQ ID NOs in the Sequence Listing (*e.g.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7....).

In another preferred embodiment, the isolated nucleic acid molecule is derived from *C. glutamicum* and encodes a protein (*e.g.*, an SMP fusion protein) which includes a biologically active domain which is at least about 50% or more homologous to one of the amino acid sequences of the invention (*e.g.*, a sequence of one of the even-numbered SEQ ID NOs in the Sequence Listing) and is able to perform a function involved in the metabolism of carbon compounds such as sugars or the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*, or has one or more of the activities set forth in Table 1, and which also includes heterologous nucleic acid sequences encoding a heterologous polypeptide or regulatory regions.

In another embodiment, the isolated nucleic acid molecule is at least 15 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of the invention (*e.g.*, a sequence of an odd-numbered SEQ ID NO in the Sequence Listing) A. Preferably, the isolated nucleic acid molecule corresponds to a naturally-occurring nucleic acid molecule. More preferably, the isolated nucleic acid encodes a naturally-occurring *C. glutamicum* SMP protein, or a biologically active portion thereof.

Another aspect of the invention pertains to vectors, *e.g.*, recombinant expression vectors, containing the nucleic acid molecules of the invention, and host cells into which such vectors have been introduced. In one embodiment, such a host cell is used to produce an SMP protein by culturing the host cell in a suitable medium. The SMP protein can be then isolated from the medium or the host cell.

Yet another aspect of the invention pertains to a genetically altered microorganism in which an SMP gene has been introduced or altered. In one embodiment, the genome of the microorganism has been altered by introduction of a nucleic acid molecule of the invention encoding wild-type or mutated SMP sequence as a transgene. In another embodiment, an endogenous SMP gene within the genome of the microorganism has been altered, *e.g.*, functionally disrupted, by homologous recombination with an altered SMP gene. In another embodiment, an endogenous or introduced SMP gene in a microorganism has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional SMP protein. In still another embodiment, one or more of the regulatory regions (*e.g.*, a promoter, repressor, or inducer) of an SMP gene in a microorganism has been altered (*e.g.*, by deletion, truncation, inversion, or point mutation) such that the expression of the SMP gene is modulated. In a preferred embodiment, the microorganism belongs to the genus *Corynebacterium* or *Brevibacterium*, with *Corynebacterium glutamicum* being particularly preferred. In a preferred embodiment, the microorganism is also utilized for the production of a desired compound, such as an amino acid, with lysine being particularly preferred.

In another aspect, the invention provides a method of identifying the presence or activity of *Cornyebacterium diphtheriae* in a subject. This method includes detection of one or more of the nucleic acid or amino acid sequences of the invention (*e.g.*, the

sequences set forth in the Sequence Listing as SEQ ID NOs 1 through 782) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject.

Still another aspect of the invention pertains to an isolated SMP protein or a portion, *e.g.*, a biologically active portion, thereof. In a preferred embodiment, the isolated SMP protein or portion thereof is capable of performing a function involved in the metabolism of carbon compounds such as sugars or in the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. In another preferred embodiment, the isolated SMP protein or portion thereof is sufficiently homologous to an amino acid sequence of the invention (*e.g.*, a sequence of an even-numbered SEQ ID NO: in the Sequence Listing) such that the protein or portion thereof maintains the ability to perform a function involved in the metabolism of carbon compounds such as sugars or in the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*.

The invention also provides an isolated preparation of an SMP protein. In preferred embodiments, the SMP protein comprises an amino acid sequence of the invention (*e.g.*, a sequence of an even-numbered SEQ ID NO: of the Sequence Listing). In another preferred embodiment, the invention pertains to an isolated full length protein which is substantially homologous to an entire amino acid sequence of the invention (*e.g.*, a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) (encoded by an open reading frame set forth in a corresponding odd-numbered SEQ ID NO: of the Sequence Listing). In yet another embodiment, the protein is at least about 50%, preferably at least about 60%, and more preferably at least about 70%, 80%, or 90%, and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an entire amino acid sequence of the invention (*e.g.*, a sequence of an even-numbered SEQ ID NO: of the Sequence Listing). In other embodiments, the isolated SMP protein comprises an amino acid sequence which is at least about 50% or more homologous to one of the amino acid sequences of the invention (*e.g.*, a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) and is able to perform a function involved in the metabolism of carbon compounds such as sugars or in the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*, or has one or more of the activities set forth in Table 1.

Alternatively, the isolated SMP protein can comprise an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, *e.g.*, hybridizes under stringent conditions, or is at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80%, or 90%, and even more preferably at least about 95%, 96%, 97%, 98,%, or 99% or more homologous to a nucleotide sequence of one of the even-numbered SEQ ID NOs set forth in the Sequence Listing. It is also preferred that the preferred forms of SMP proteins also have one or more of the SMP bioactivities described herein.

The SMP polypeptide, or a biologically active portion thereof, can be operatively linked to a non-SMP polypeptide to form a fusion protein. In preferred embodiments, this fusion protein has an activity which differs from that of the SMP protein alone. In other preferred embodiments, this fusion protein performs a function involved in the metabolism of carbon compounds such as sugars or in the generation of energy molecules (*e.g.*, ATP) by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. In particularly preferred embodiments, integration of this fusion protein into a host cell modulates production of a desired compound from the cell.

In another aspect, the invention provides methods for screening molecules which modulate the activity of an SMP protein, either by interacting with the protein itself or a substrate or binding partner of the SMP protein, or by modulating the transcription or translation of an SMP nucleic acid molecule of the invention.

Another aspect of the invention pertains to a method for producing a fine chemical. This method involves the culturing of a cell containing a vector directing the expression of an SMP nucleic acid molecule of the invention, such that a fine chemical is produced. In a preferred embodiment, this method further includes the step of obtaining a cell containing such a vector, in which a cell is transfected with a vector directing the expression of an SMP nucleic acid. In another preferred embodiment, this method further includes the step of recovering the fine chemical from the culture. In a particularly preferred embodiment, the cell is from the genus *Corynebacterium* or *Brevibacterium*, or is selected from those strains set forth in Table 3.

Another aspect of the invention pertains to methods for modulating production of a molecule from a microorganism. Such methods include contacting the cell with an

agent which modulates SMP protein activity or SMP nucleic acid expression such that a cell associated activity is altered relative to this same activity in the absence of the agent. In a preferred embodiment, the cell is modulated for one or more *C. glutamicum* carbon metabolism pathways or for the production of energy through processes such as oxidative phosphorylation, such that the yields or rate of production of a desired fine chemical by this microorganism is improved. The agent which modulates SMP protein activity can be an agent which stimulates SMP protein activity or SMP nucleic acid expression. Examples of agents which stimulate SMP protein activity or SMP nucleic acid expression include small molecules, active SMP proteins, and nucleic acids encoding SMP proteins that have been introduced into the cell. Examples of agents which inhibit SMP activity or expression include small molecules and antisense SMP nucleic acid molecules.

Another aspect of the invention pertains to methods for modulating yields of a desired compound from a cell, involving the introduction of a wild-type or mutant SMP gene into a cell, either maintained on a separate plasmid or integrated into the genome of the host cell. If integrated into the genome, such integration can be random, or it can take place by homologous recombination such that the native gene is replaced by the introduced copy, causing the production of the desired compound from the cell to be modulated. In a preferred embodiment, said yields are increased. In another preferred embodiment, said chemical is a fine chemical. In a particularly preferred embodiment, said fine chemical is an amino acid. In especially preferred embodiments, said amino acid is L-lysine.

Detailed Description of the Invention

The present invention provides SMP nucleic acid and protein molecules which are involved in the metabolism of carbon compounds such as sugars and the generation of energy molecules by processes such as oxidative phosphorylation in *Corynebacterium glutamicum*. The molecules of the invention may be utilized in the modulation of production of fine chemicals from microorganisms, such as *C. glutamicum*, either directly (*e.g.*, where overexpression or optimization of a glycolytic pathway protein has a direct impact on the yield, production, and/or efficiency of production of, *e.g.*, pyruvate from modified *C. glutamicum*), or may have an indirect

impact which nonetheless results in an increase of yield, production, and/or efficiency of production of the desired compound (*e.g.*, where modulation of proteins involved in oxidative phosphorylation results in alterations in the amount of energy available to perform necessary metabolic processes and other cellular functions, such as nucleic acid and protein biosynthesis and transcription/translation). Aspects of the invention are further explicated below.

I. Fine Chemicals

The term 'fine chemical' is art-recognized and includes molecules produced by an organism which have applications in various industries, such as, but not limited to, the pharmaceutical, agriculture, and cosmetics industries. Such compounds include organic acids, such as tartaric acid, itaconic acid, and diaminopimelic acid, both proteinogenic and non-proteinogenic amino acids, purine and pyrimidine bases, nucleosides, and nucleotides (as described *e.g.* in Kuninaka, A. (1996) Nucleotides and related compounds, p. 561-612, in Biotechnology vol. 6, Rehm *et al.*, eds. VCH: Weinheim, and references contained therein), lipids, both saturated and unsaturated fatty acids (*e.g.*, arachidonic acid), diols (*e.g.*, propane diol, and butane diol), carbohydrates (*e.g.*, hyaluronic acid and trehalose), aromatic compounds (*e.g.*, aromatic amines, vanillin, and indigo), vitamins and cofactors (as described in Ullmann's Encyclopedia of Industrial Chemistry, vol. A27, "Vitamins", p. 443-613 (1996) VCH: Weinheim and references therein; and Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research – Asia, held Sept. 1-3, 1994 at Penang, Malaysia, AOCS Press, (1995)), enzymes, polyketides (Cane *et al.* (1998) *Science* 282: 63-68), and all other chemicals described in Gutcho (1983) Chemicals by Fermentation, Noyes Data Corporation, ISBN: 0818805086 and references therein. The metabolism and uses of certain of these fine chemicals are further explicated below.

A. *Amino Acid Metabolism and Uses*

Amino acids comprise the basic structural units of all proteins, and as such are essential for normal cellular functioning in all organisms. The term "amino acid" is art-

recognized. The proteinogenic amino acids, of which there are 20 species, serve as structural units for proteins, in which they are linked by peptide bonds, while the nonproteinogenic amino acids (hundreds of which are known) are not normally found in proteins (see Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97 VCH: Weinheim (1985)). Amino acids may be in the D- or L- optical configuration, though L-amino acids are generally the only type found in naturally-occurring proteins. Biosynthetic and degradative pathways of each of the 20 proteinogenic amino acids have been well characterized in both prokaryotic and eukaryotic cells (see, for example, Stryer, L. Biochemistry, 3rd edition, pages 578-590 (1988)). The 'essential' amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), so named because they are generally a nutritional requirement due to the complexity of their biosyntheses, are readily converted by simple biosynthetic pathways to the remaining 11 'nonessential' amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine). Higher animals do retain the ability to synthesize some of these amino acids, but the essential amino acids must be supplied from the diet in order for normal protein synthesis to occur.

Aside from their function in protein biosynthesis, these amino acids are interesting chemicals in their own right, and many have been found to have various applications in the food, feed, chemical, cosmetics, agriculture, and pharmaceutical industries. Lysine is an important amino acid in the nutrition not only of humans, but also of monogastric animals such as poultry and swine. Glutamate is most commonly used as a flavor additive (mono-sodium glutamate, MSG) and is widely used throughout the food industry, as are aspartate, phenylalanine, glycine, and cysteine. Glycine, L-methionine and tryptophan are all utilized in the pharmaceutical industry. Glutamine, valine, leucine, isoleucine, histidine, arginine, proline, serine and alanine are of use in both the pharmaceutical and cosmetics industries. Threonine, tryptophan, and D/ L-methionine are common feed additives. (Leuchtenberger, W. (1996) Amino aids – technical production and use, p. 466-502 in Rehm *et al.* (eds.) Biotechnology vol. 6, chapter 14a, VCH: Weinheim). Additionally, these amino acids have been found to be useful as precursors for the synthesis of synthetic amino acids and proteins, such as N-acetylcysteine, S-carboxymethyl-L-cysteine, (S)-5-hydroxytryptophan, and others

described in Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97, VCH: Weinheim, 1985.

The biosynthesis of these natural amino acids in organisms capable of producing them, such as bacteria, has been well characterized (for review of bacterial amino acid biosynthesis and regulation thereof, see Umbarger, H.E.(1978) *Ann. Rev. Biochem.* 47: 533-606). Glutamate is synthesized by the reductive amination of α-ketoglutarate, an intermediate in the citric acid cycle. Glutamine, proline, and arginine are each subsequently produced from glutamate. The biosynthesis of serine is a three-step process beginning with 3-phosphoglycerate (an intermediate in glycolysis), and resulting in this amino acid after oxidation, transamination, and hydrolysis steps. Both cysteine and glycine are produced from serine; the former by the condensation of homocysteine with serine, and the latter by the transferal of the side-chain β-carbon atom to tetrahydrofolate, in a reaction catalyzed by serine transhydroxymethylase. Phenylalanine, and tyrosine are synthesized from the glycolytic and pentose phosphate pathway precursors erythrose 4-phosphate and phosphoenolpyruvate in a 9-step biosynthetic pathway that differ only at the final two steps after synthesis of prephenate. Tryptophan is also produced from these two initial molecules, but its synthesis is an 11-step pathway. Tyrosine may also be synthesized from phenylalanine, in a reaction catalyzed by phenylalanine hydroxylase. Alanine, valine, and leucine are all biosynthetic products of pyruvate, the final product of glycolysis. Aspartate is formed from oxaloacetate, an intermediate of the citric acid cycle. Asparagine, methionine, threonine, and lysine are each produced by the conversion of aspartate. Isoleucine is formed from threonine. A complex 9-step pathway results in the production of histidine from 5-phosphoribosyl-1-pyrophosphate, an activated sugar.

Amino acids in excess of the protein synthesis needs of the cell cannot be stored, and are instead degraded to provide intermediates for the major metabolic pathways of the cell (for review see Stryer, L. Biochemistry 3rd ed. Ch. 21 "Amino Acid Degradation and the Urea Cycle" p. 495-516 (1988)). Although the cell is able to convert unwanted amino acids into useful metabolic intermediates, amino acid production is costly in terms of energy, precursor molecules, and the enzymes necessary to synthesize them. Thus it is not surprising that amino acid biosynthesis is regulated by feedback inhibition, in which the presence of a particular amino acid serves to slow or entirely stop its own

production (for overview of feedback mechanisms in amino acid biosynthetic pathways, see Stryer, L. Biochemistry, 3rd ed. Ch. 24: "Biosynthesis of Amino Acids and Heme" p. 575-600 (1988)). Thus, the output of any particular amino acid is limited by the amount of that amino acid present in the cell.

B. Vitamin, Cofactor, and Nutraceutical Metabolism and Uses

Vitamins, cofactors, and nutraceuticals comprise another group of molecules which the higher animals have lost the ability to synthesize and so must ingest, although they are readily synthesized by other organisms such as bacteria. These molecules are either bioactive substances themselves, or are precursors of biologically active substances which may serve as electron carriers or intermediates in a variety of metabolic pathways. Aside from their nutritive value, these compounds also have significant industrial value as coloring agents, antioxidants, and catalysts or other processing aids. (For an overview of the structure, activity, and industrial applications of these compounds, see, for example, Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996.) The term "vitamin" is art-recognized, and includes nutrients which are required by an organism for normal functioning, but which that organism cannot synthesize by itself. The group of vitamins may encompass cofactors and nutraceutical compounds. The language "cofactor" includes nonproteinaceous compounds required for a normal enzymatic activity to occur. Such compounds may be organic or inorganic; the cofactor molecules of the invention are preferably organic. The term "nutraceutical" includes dietary supplements having health benefits in plants and animals, particularly humans. Examples of such molecules are vitamins, antioxidants, and also certain lipids (*e.g.*, polyunsaturated fatty acids).

The biosynthesis of these molecules in organisms capable of producing them, such as bacteria, has been largely characterized (Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley & Sons; Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological

methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave SMP mRNA transcripts to thereby inhibit translation of SMP mRNA. A ribozyme having specificity for an SMP-encoding nucleic acid can be designed based upon the nucleotide sequence of an SMP cDNA disclosed herein (*i.e.*, SEQ ID NO. 3 (RXA01626)). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an SMP-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071 and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, SMP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, SMP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of an SMP nucleotide sequence (*e.g.*, an SMP promoter and/or enhancers) to form triple helical structures that prevent transcription of an SMP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

B. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an SMP protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of

autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells. Preferred regulatory sequences are, for example, promoters such as cos-, tac-, trp-, tet-, trp-tet-, lpp-, lac-, lpp-lac-, lacI^q-, T7-, T5-, T3-, gal-, trc-, ara-, SP6-, arny, SPO2, λ-P_R- or λ P_L, which are used preferably in bacteria. Additional regulatory sequences are, for example, promoters from yeasts and fungi, such as ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH, promoters from plants such as CaMV/35S, SSU, OCS, lib4,

usp, STLS1, B33, nos or ubiquitin- or phaseolin-promoters. It is also possible to use artificial promoters. It will be appreciated by those of ordinary skill in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, SMP proteins, mutant forms of SMP proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of SMP proteins in prokaryotic or eukaryotic cells. For example, SMP genes can be expressed in bacterial cells such as *C. glutamicum*, insect cells (using baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A. *et al.* (1992) "Foreign gene expression in yeast: a review", *Yeast* 8: 423-488; van den Hondel, C.A.M.J.J. *et al.* (1991) "Heterologous gene expression in filamentous fungi" in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F. *et al.*, eds., p. 1-28, Cambridge University Press: Cambridge), algae and multicellular plant cells (see Schmidt, R. and Willmitzer, L. (1988) High efficiency *Agrobacterium tumefaciens* –mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" *Plant Cell Rep*: 583-586), or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein but also to the C-terminus or fused within suitable regions in the proteins. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion

expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. In one embodiment, the coding sequence of the SMP protein is cloned into a pGEX expression vector to create a vector encoding a fusion protein comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-X protein. The fusion protein can be purified by affinity chromatography using glutathione-agarose resin. Recombinant SMP protein unfused to GST can be recovered by cleavage of the fusion protein with thrombin.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315), pLG338, pACYC184, pBR322, pUC18, pUC19, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11, pBdCl, and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89; and Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. For transformation of other varieties of bacteria, appropriate vectors may be selected. For example, the plasmids pIJ101, pIJ364, pIJ702 and pIJ361 are known to be useful in transforming Streptomyces, while plasmids pUB110, pC194, or pBD214 are suited for transformation of Bacillus species. Several plasmids of use in the transfer of genetic information into Corynebacterium include pHM1519, pBL1, pSA77, or pAJ667 (Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018).

One strategy to maximize recombinant protein expression is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as *C. glutamicum* (Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the SMP protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), 2 μ, pAG-1, Yep6, Yep13, pEMBLYe23, pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for the construction of vectors appropriate for use in other fungi, such as the filamentous fungi, include those detailed in: van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, J.F. Peberdy, *et al.*, eds., p. 1-28, Cambridge University Press: Cambridge, and Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York (IBSN 0 444 904018).

Alternatively, the SMP proteins of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In another embodiment, the SMP proteins of the invention may be expressed in unicellular plant cells (such as algae) or in plant cells from higher plants (*e.g.*, the spermatophytes, such as crop plants). Examples of plant expression vectors include those detailed in: Becker, D., Kemper, E., Schell, J. and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", *Plant Mol. Biol.* 20: 1195-1197; and Bevan, M.W. (1984) "Binary *Agrobacterium* vectors for plant transformation", *Nucl. Acid. Res.* 12: 8711-8721, and include pLGV23, pGHlac+,

pBIN19, pAK2004, and pDH51 (Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in

a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to SMP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an SMP protein can be expressed in bacterial cells such as *C. glutamicum*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to one of ordinary skill in the art. Microorganisms related to *Corynebacterium glutamicum* which may be conveniently used as host cells for the nucleic acid and protein molecules of the invention are set forth in Table 3.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection", "conjugation" and "transduction" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, linear DNA or RNA (*e.g.*, a linearized vector or a gene construct alone without a vector) or nucleic acid in the form of a vector (*e.g.*, a plasmid, phage, phasmid, phagemid,

transposon or other DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemical-mediated transfer, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an SMP protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by, for example, drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

To create a homologous recombinant microorganism, a vector is prepared which contains at least a portion of an SMP gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the SMP gene. Preferably, this SMP gene is a *Corynebacterium glutamicum* SMP gene, but it can be a homologue from a related bacterium or even from a mammalian, yeast, or insect source. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous SMP gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous SMP gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous SMP protein). In the homologous recombination vector, the altered portion of the SMP gene is flanked at its 5' and 3' ends by additional nucleic acid of the SMP gene to allow for homologous recombination to occur between the exogenous SMP gene carried by the vector and an endogenous SMP gene in a microorganism. The additional

flanking SMP nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see *e.g.*, Thomas, K.R., and Capecchi, M.R. (1987) Cell 51: 503 for a description of homologous recombination vectors). The vector is introduced into a microorganism (*e.g.*, by electroporation) and cells in which the introduced SMP gene has homologously recombined with the endogenous SMP gene are selected, using art-known techniques.

In another embodiment, recombinant microorganisms can be produced which contain selected systems which allow for regulated expression of the introduced gene. For example, inclusion of an SMP gene on a vector placing it under control of the lac operon permits expression of the SMP gene only in the presence of IPTG. Such regulatory systems are well known in the art.

In another embodiment, an endogenous SMP gene in a host cell is disrupted (*e.g.*, by homologous recombination or other genetic means known in the art) such that expression of its protein product does not occur. In another embodiment, an endogenous or introduced SMP gene in a host cell has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional SMP protein. In still another embodiment, one or more of the regulatory regions (*e.g.*, a promoter, repressor, or inducer) of an SMP gene in a microorganism has been altered (*e.g.*, by deletion, truncation, inversion, or point mutation) such that the expression of the SMP gene is modulated. One of ordinary skill in the art will appreciate that host cells containing more than one of the described SMP gene and protein modifications may be readily produced using the methods of the invention, and are meant to be included in the present invention.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an SMP protein. Accordingly, the invention further provides methods for producing SMP proteins using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding an SMP protein has been introduced, or into which genome has been introduced a gene encoding a wild-type or altered SMP protein) in a suitable medium until SMP protein is produced. In another

embodiment, the method further comprises isolating SMP proteins from the medium or the host cell.

C. Isolated SMP Proteins

Another aspect of the invention pertains to isolated SMP proteins, and biologically active portions thereof. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of SMP protein in which the protein is separated from cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of SMP protein having less than about 30% (by dry weight) of non-SMP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-SMP protein, still more preferably less than about 10% of non-SMP protein, and most preferably less than about 5% non-SMP protein. When the SMP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of SMP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of SMP protein having less than about 30% (by dry weight) of chemical precursors or non-SMP chemicals, more preferably less than about 20% chemical precursors or non-SMP chemicals, still more preferably less than about 10% chemical precursors or non-SMP chemicals, and most preferably less than about 5% chemical precursors or non-SMP chemicals. In preferred embodiments, isolated proteins or biologically active portions thereof lack contaminating proteins from the same organism from which the SMP protein is derived. Typically, such proteins are produced by recombinant expression of, for example, a *C. glutamicum* SMP protein in a microorganism such as *C. glutamicum*.

frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify SMP homologues (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In another embodiment, cell based assays can be exploited to analyze a variegated SMP library, using methods well known in the art.

D. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, fusion proteins, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *C. glutamicum* and related organisms; mapping of genomes of organisms related to *C. glutamicum*; identification and localization of *C. glutamicum* sequences of interest; evolutionary studies; determination of SMP protein regions required for function; modulation of an SMP protein activity; modulation of the metabolism of one or more sugars; modulation of high-energy molecule production in a cell (*i.e.*, ATP, NADPH); and modulation of cellular production of a desired compound, such as a fine chemical.

The SMP nucleic acid molecules of the invention have a variety of uses. First, they may be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof. Also, they may be used to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is nonpathogenic, it is related to pathogenic species, such as *Corynebacterium diphtheriae*. *Corynebacterium diphtheriae* is the causative agent of diphtheria, a rapidly developing, acute, febrile infection which involves both local and systemic pathology. In this disease, a local lesion develops in the upper respiratory tract and involves necrotic injury to epithelial cells; the bacilli secrete toxin which is disseminated through this lesion to distal susceptible tissues of the body. Degenerative changes brought about by the inhibition of protein synthesis in these tissues, which include heart, muscle, peripheral nerves, adrenals, kidneys, liver and

spleen, result in the systemic pathology of the disease. Diphtheria continues to have high incidence in many parts of the world, including Africa, Asia, Eastern Europe and the independent states of the former Soviet Union. An ongoing epidemic of diphtheria in the latter two regions has resulted in at least 5,000 deaths since 1990.

In one embodiment, the invention provides a method of identifying the presence or activity of *Cornyebacterium diphtheriae* in a subject. This method includes detection of one or more of the nucleic acid or amino acid sequences of the invention (*e.g.*, the sequences set forth as odd-numbered or even-numbered SEQ ID NOs, respectively, in the Sequence Listing) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject. *C. glutamicum* and *C. diphtheriae* are related bacteria, and many of the nucleic acid and protein molecules in C. *glutamicum* are homologous to *C. diphtheriae* nucleic acid and protein molecules, and can therefore be used to detect *C. diphtheriae* in a subject.

The nucleic acid and protein molecules of the invention may also serve as markers for specific regions of the genome. This has utility not only in the mapping of the genome, but also for functional studies of *C. glutamicum* proteins. For example, to identify the region of the genome to which a particular *C. glutamicum* DNA-binding protein binds, the *C. glutamicum* genome could be digested, and the fragments incubated with the DNA-binding protein. Those which bind the protein may be additionally probed with the nucleic acid molecules of the invention, preferably with readily detectable labels; binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map of *C. glutamicum*, and, when performed multiple times with different enzymes, facilitates a rapid determination of the nucleic acid sequence to which the protein binds. Further, the nucleic acid molecules of the invention may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related bacteria, such as *Brevibacterium lactofermentum*.

The SMP nucleic acid molecules of the invention are also useful for evolutionary and protein structural studies. The metabolic and energy-releasing processes in which the molecules of the invention participate are utilized by a wide variety of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the

evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the protein which are essential for the functioning of the enzyme. This type of determination is of value for protein engineering studies and may give an indication of what the protein can tolerate in terms of mutagenesis without losing function.

Manipulation of the SMP nucleic acid molecules of the invention may result in the production of SMP proteins having functional differences from the wild-type SMP proteins. These proteins may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

The invention provides methods for screening molecules which modulate the activity of an SMP protein, either by interacting with the protein itself or a substrate or binding partner of the SMP protein, or by modulating the transcription or translation of an SMP nucleic acid molecule of the invention. In such methods, a microorganism expressing one or more SMP proteins of the invention is contacted with one or more test compounds, and the effect of each test compound on the activity or level of expression of the SMP protein is assessed.

There are a number of mechanisms by which the alteration of an SMP protein of the invention may directly affect the yield, production, and/or efficiency of production of a fine chemical from a *C. glutamicum* strain incorporating such an altered protein. The degradation of high-energy carbon molecules such as sugars, and the conversion of compounds such as NADH and $FADH_2$ to more useful forms via oxidative phosphorylation results in a number of compounds which themselves may be desirable fine chemicals, such as pyruvate, ATP, NADH, and a number of intermediate sugar compounds. Further, the energy molecules (such as ATP) and the reducing equivalents (such as NADH or NADPH) produced by these metabolic pathways are utilized in the cell to drive reactions which would otherwise be energetically unfavorable. Such unfavorable reactions include many biosynthetic pathways for fine chemicals. By improving the ability of the cell to utilize a particular sugar (*e.g.*, by manipulating the genes encoding enzymes involved in the degradation and conversion of that sugar into energy for the cell), one may increase the amount of energy available to permit

unfavorable, yet desired metabolic reactions (*e.g.*, the biosynthesis of a desired fine chemical) to occur.

Further, modulation of one or more pathways involved in sugar utilization permits optimization of the conversion of the energy contained within the sugar molecule to the production of one or more desired fine chemicals. For example, by reducing the activity of enzymes involved in, for example, gluconeogenesis, more ATP is available to drive desired biochemical reactions (such as fine chemical biosyntheses) in the cell. Also, the overall production of energy molecules from sugars may be modulated to ensure that the cell maximizes its energy production from each sugar molecule. Inefficient sugar utilization can lead to excess CO_2 production and excess energy, which may result in futile metabolic cycles. By improving the metabolism of sugar molecules, the cell should be able to function more efficiently, with a need for fewer carbon molecules. This should result in an improved fine chemical product: sugar molecule ratio (improved carbon yield), and permits a decrease in the amount of sugars that must be added to the medium in large-scale fermentor culture of such engineered *C. glutamicum*.

The mutagenesis of one or more SMP genes of the invention may also result in SMP proteins having altered activities which indirectly impact the production of one or more desired fine chemicals from *C. glutamicum*. For example, by increasing the efficiency of utilization of one or more sugars (such that the conversion of the sugar to useful energy molecules is improved), or by increasing the efficiency of conversion of reducing equivalents to useful energy molecules (*e.g.*, by improving the efficiency of oxidative phosphorylation, or the activity of the ATP synthase), one can increase the amount of these high-energy compounds available to the cell to drive normally unfavorable metabolic processes. These processes include the construction of cell walls, transcription, translation, and the biosynthesis of compounds necessary for growth and division of the cells (*e.g.*, nucleotides, amino acids, vitamins, lipids, etc.) (Lengeler *et al.* (1999) Biology of Prokaryotes, Thieme Verlag: Stuttgart, p. 88-109; 913-918; 875-899). By improving the growth and multiplication of these engineered cells, it is possible to increase both the viability of the cells in large-scale culture, and also to improve their rate of division, such that a relatively larger number of cells can survive in fermentor culture. The yield, production, or efficiency of production may be increased, at least

due to the presence of a greater number of viable cells, each producing the desired fine chemical.

Further, many of the degradation products produced during sugar metabolism are themselves utilized by the cell as precursors or intermediates for the production of a number of other useful compounds, some of which are fine chemicals. For example, pyruvate is converted into the amino acid alanine, and ribose-5-phosphate is an integral part of, for example, nucleotide molecules. The amount and efficiency of sugar metabolism, then, has a profound effect on the availability of these degradation products in the cell. By increasing the ability of the cell to process sugars, either in terms of efficiency of existing pathways (*e.g.*, by engineering enzymes involved in these pathways such that they are optimized in activity), or by increasing the availability of the enzymes involved in such pathways (*e.g.*, by increasing the number of these enzymes present in the cell), it is possible to also increase the availability of these degradation products in the cell, which should in turn increase the production of many different other desirable compounds in the cell (*e.g.*, fine chemicals).

The aforementioned mutagenesis strategies for SMP proteins to result in increased yields of a fine chemical from *C. glutamicum* are not meant to be limiting; variations on these strategies will be readily apparent to one of ordinary skill in the art. Using such strategies, and incorporating the mechanisms disclosed herein, the nucleic acid and protein molecules of the invention may be utilized to generate *C. glutamicum* or related strains of bacteria expressing mutated SMP nucleic acid and protein molecules such that the yield, production, and/or efficiency of production of a desired compound is improved. This desired compound may be any product produced by *C. glutamicum*, which includes the final products of biosynthesis pathways and intermediates of naturally-occurring metabolic pathways, as well as molecules which do not naturally occur in the metabolism of *C. glutamicum*, but which are produced by a *C. glutamicum* strain of the invention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patent applications, patents, published patent applications, Tables, and the sequence listing cited throughout this application are hereby incorporated by reference.

TABLE 1: GENES IN THE APPLICATION

HMP:

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
1	2	RXS02735	VV0074	14576	15280	6-Phosphoglucolactonase
3	4	RXA01626	GR00452	4270	3926	L-ribulose-phosphate 4-epimerase
5	6	RXA02245	GR00654	13639	14296	RIBULOSE-PHOSPHATE 3-EPIMERASE (EC 5.1.3.1)
7	8	RXA01015	GR00290	348	5	RIBOSE 5-PHOSPHATE ISOMERASE (EC 5.3.1.6)

TCA:

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
9	10	RXN01312	VV0082	20603	18785	SUCCINATE DEHYDROGENASE FLAVOPROTEIN SUBUNIT (EC 1.3.99.1)
11	12	F RXA01312	GR00380	2690	1814	SUCCINATE DEHYDROGENASE FLAVOPROTEIN SUBUNIT (EC 1.3.99.1)
13	14	RXN00231	VV0083	15484	14015	SUCCINATE-SEMIALDEHYDE DEHYDROGENASE (NADP+) (EC 1.2.1.16)
15	16	RXA01311	GR00380	1611	855	SUCCINATE DEHYDROGENASE IRON-SULFUR PROTEIN (EC 1.3.99.1)
17	18	RXA01535	GR00427	1354	2760	FUMARATE HYDRATASE PRECURSOR (EC 4.2.1.2)
19	20	RXA00517	GR00131	1407	2447	MALATE DEHYDROGENASE (EC 1.1.1.37) (EC 1.1.1.82)
21	22	RXA01350	GR00392	1844	2827	MALATE DEHYDROGENASE (EC 1.1.1.37)

EMB-Pathway

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
23	24	RXA02149	GR00639	17786	18754	GLUCOKINASE (EC 2.7.1.2)
25	26	RXA01814	GR00515	2571	910	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
27	28	RXN02803	VV0086	1	657	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
29	30	F RXA02803	GR00784	2	400	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
31	32	RXN03076	VV0043	1624	35	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
33	34	F RXA02854	GR10002	1588	5	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
35	36	RXA00511	GR00129	1	513	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig	NT Start	NT Stop	Function
		RXN01365	VV0091	1476	103	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE (EC 5.4.2.8)
37	38					PHOSPHOGLUCOMUTASE (EC 5.4.2.2) / PHOSPHOMANNOMUTASE
		F RXA01365	GR00397	897	4	(EC 5.4.2.8)
39	40					GLUCOSE-6-PHOSPHATE ISOMERASE (GPI) (EC 5.3.1.9)
		RXA00098	GR00014	8525	8144	GLUCOSE-6-PHOSPHATE ISOMERASE A (GPI A) (EC 5.3.1.9)
41	42	RXA01988	GR00578	1	630	GLUCOSE-6-PHOSPHATE MUTASE (EC 5.4.2.1)
43	44	RXA00340	GR00059	1549	2694	PHOSPHOGLYCERATE MUTASE (EC 5.4.2.1)
45	46	RXA02492	GR00720	2201	2917	PHOSPHOGLYCERATE MUTASE (EC 5.4.2.1)
47	48	RXA00381	GR00082	1451	846	PHOSPHOGLYCERATE MUTASE (EC 5.4.2.1)
49	50	RXA02122	GR00836	6511	5813	PHOSPHOFRUCTOKINASE (EC 2.7.1.11)
51	52	RXA00206	GR00032	6171	5134	6-PHOSPHOFRUCTOKINASE (EC 2.7.1.56)
53	54	RXA01243	GR00359	2302	3261	1-PHOSPHOFRUCTOKINASE (EC 2.7.1.56)
55	56	RXA01882	GR01538	1165	2154	1-PHOSPHOFRUCTOKINASE ALDOLASE (EC 4.1.2.13)
57	58	RXA01702	GR00479	1397	366	FRUCTOSE-BISPHOSPHATE ISOMERASE (EC 5.3.1.1)
59	60	RXA02258	GR00654	28451	27227	TRIOSEPHOSPHATE DEHYDROGENASE (EC 1.2.1.12)
61	62	RXN01225	VV0064	6382	4943	3-PHOSPHATE DEHYDROGENASE HOMOLOG
63	64	F RXA01225	GR00354	5302	6741	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12)
65	66	RXA02256	GR00654	23934	24935	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12)
67	68	RXA02257	GR00654	25155	26369	PHOSPHOGLYCERATE KINASE (EC 2.7.2.3)
69	70	RXA00235	GR00036	2365	1091	ENOLASE (EC 4.2.1.11)
71	72	RXA01093	GR00306	1552	122	PYRUVATE KINASE (EC 2.7.1.40)
73	74	RXN02675	VV0098	72801	70945	PYRUVATE KINASE (EC 2.7.1.40)
75	76	F RXA02675	GR00764	2	384	PYRUVATE KINASE (EC 2.7.1.40)
77	78	F RXA02696	GR00755	2849	4370	PYRUVATE SYNTHASE (EC 2.7.9.2)
79	80	RXA00682	GR00179	5299	3401	PHOSPHOENOLPYRUVATE SYNTHASE (EC 2.7.9.2)
81	82	RXA00683	GR00179	6440	5349	PHOSPHOENOLPYRUVATE SYNTHASE (EC 2.7.9.2)
83	84	RXN00635	VV0135	22708	20972	PYRUVATE DEHYDROGENASE (CYTOCHROME) (EC 1.2.2.2)
85	86	F RXA02807	GR00788	88	552	PYRUVATE DEHYDROGENASE (CYTOCHROME) (EC 1.2.2.2)
87	88	F RXA00635	GR00167	3	923	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
89	90	RXN03044	VV0019	1391	2221	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
91	92	F RXA02852	GR00852	3	281	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
93	94	F RXA00268	GR00041	126	965	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
95	96	RXN03088	VV0049	2243	2650	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
97	98	F RXA02887	GR10022	411	4	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
99	100	RXN03043	VV0019	1	1362	PYRUVATE DEHYDROGENASE (EC 1.8.1.4)
101	102	F RXA02897	GR10039	1291	5	DIHYDROLIPOAMIDE DEHYDROGENASE (EC 1.8.1.4)
103	104	RXN03083	VV0047	88	1110	DIHYDROLIPOAMIDE DEHYDROGENASE CARBOXYLASE (EC 4.1.1.31)
105	106	F RXA02853	GR10001	89	1486	PHOSPHOENOLPYRUVATE CARBOXYLASE (EC 6.4.1.1)
107	108	RXA02259	GR00654	27401	30172	PYRUVATE CARBOXYLASE
109	110	RXN02326	VV0047	4500	5315	PYRUVATE CARBOXYLASE (EC 6.4.1.1)
111	112	F RXA02326	GR00668	5338	4523	PYRUVATE CARBOXYLASE
113	114	RXN02327	VV0047	6305	5346	PYRUVATE CARBOXYLASE (EC 6.4.1.1)
115	116	F RXA02327	GR00668	1842	3437	PYRUVATE CARBOXYLASE (EC 6.4.1.1)
117	118	RXN02328	VV0047	7783	6401	MALIC ENZYME (EC 1.1.1.39)
119	120	F RXA02328	GR00668	12539	11316	
121	122	RXN01048	VV0079			
123	124					

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
125	126	F RXA01048	GR00296	3	290	MALIC ENZYME (EC 1.1.1.39)
127	128	F RXA00290	GR00046	4693	5655	MALIC ENZYME (EC 1.1.1.39)
129	130	RXA02694	GR00755	1879	2820	L-LACTATE DEHYDROGENASE (EC 1.1.1.27)
131	132	RXN00296	VV0176	35783	38606	D-LACTATE DEHYDROGENASE (CYTOCHROME) (EC 1.1.2.4)
133	134	F RXA00296	GR00046	3	2837	D-LACTATE DEHYDROGENASE (CYTOCHROME) (EC 1.1.2.4)
135	136	RXA01901	GR00544	4156	5417	L-LACTATE DEHYDROGENASE (CYTOCHROME) (EC 1.1.2.3)
137	138	RXN01952	VV0105	9954	11666	L-LACTATE DEHYDROGENASE (EC 1.1.1.28)
139	140	F RXA01952	GR00562	1	216	D-LACTATE DEHYDROGENASE (EC 1.1.1.28)
141	142	F RXA01955	GR00562	4611	6209	D-LACTATE DEHYDROGENASE (EC 1.1.1.28)
143	144	RXA00293	GR00047	2645	1734	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
145	146	RXN01130	VV0157	6138	5536	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
147	148	F RXA01130	GR00315	2	304	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
149	150	RXN03112	VV0085	509	6	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
151	152	F RXA01133	GR00316	588	1116	IOLB PROTEIN
153	154	RXN00871	VV0127	3127	2240	IOLB PROTEIN: D-FRUCTOSE 1,6-BISPHOSPHATE = GLYCERONE-CC
155	156	F RXA00871	GR00239	2344	3207	PHOSPHATE + D- GLYCERALDEHYDE 3-PHOSPHATE.
157	158	RXN02829	VV0354	287	559	IOLS PROTEIN
159	160	F RXA02829	GR00816	287	582	IOLS PROTEIN
161	162	RXN01468	VV0019	7474	8298	NAGD PROTEIN
163	164	F RXA01468	GR00422	1250	2074	PUTATIVE N-GLYCERALDEHYDE-2-PHOSPHOTRANSFERASE
165	166	RXA00794	GR00211	3993	2989	GLPX PROTEIN
167	168	RXN02920	VV0213	6135	5224	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
169	170	F RXA02379	GR00690	1390	686	D-3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)
171	172	RXN02688	VV0098	59053	58385	PHOSPHOGLYCERATE MUTASE (EC 5.4.2.1)
173	174	RXN03087	VV0052	3216	3428	PYRUVATE CARBOXYLASE (EC 6.4.1.1)
175	176	RXN03186	VV0377	310	519	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
177	178	RXN03187	VV0382	3	281	PYRUVATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.1)
179	180	RXN02591	VV0098	14370	12541	PHOSPHOENOLPYRUVATE CARBOXYKINASE [GTP] (EC 4.1.1.32)
181	182	RXS01260	VV0009	3477	2296	LIPOAMIDE DEHYDROGENASE COMPONENT (E3) OF BRANCHED-CHAIN ALPHA-KETO ACID DEHYDROGENASE COMPLEX (EC 1.8.1.4)
183	184	RXS01261	VV0009	3703	3533	LIPOAMIDE DEHYDROGENASE COMPONENT (E3) OF BRANCHED-CHAIN ALPHA-KETO ACID DEHYDROGENASE COMPLEX (EC 1.8.1.4)

Glycerol metabolism

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
185	186	RXA02640	GR00749	1400	2926	GLYCEROL KINASE (EC 2.7.1.30)
187	188	RXN01025	VV0143	5483	4488	GLYCEROL-3-PHOSPHATE DEHYDROGENASE (NAD(P)+) (EC 1.1.1.94)
189	190	F RXA01025	GR00293	939	1853	GLYCEROL-3-PHOSPHATE DEHYDROGENASE (NAD(P)+) (EC 1.1.1.94)
191	192	RXA01851	GR00525	3515	1830	AEROBIC GLYCEROL-3-PHOSPHATE DEHYDROGENASE (EC 1.1.99.5)
193	194	RXA01242	GR00359	1526	2302	GLYCEROL-3-PHOSPHATE REGULON REPRESSOR
195	196	RXA02288	GR00661	992	147	GLYCEROL-3-PHOSPHATE REGULON REPRESSOR

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
197	198	RXN01891	VV0122	24949	24086	GLYCEROL-3-PHOSPHATE-BINDING PERIPLASMIC PROTEIN PRECURSOR
199	200	F RXA01891	GR00541	1736	918	GLYCEROL-3-PHOSPHATE-BINDING PERIPLASMIC PROTEIN PRECURSOR
201	202	RXA02414	GR00703	3808	3062	Uncharacterized protein involved in glycerol metabolism (homolog of Drosophila rhomboid)
203	204	RXN01580	VV0122	22091	22807	Glycerophosphoryl diester phosphodiesterase

Acetate metabolism

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
205	206	RXA01436	GR00418	2547	1357	ACETATE KINASE (EC 2.7.2.1)
207	208	RXA00686	GR00179	8744	7941	ACETATE OPERON REPRESSOR
209	210	RXA00246	GR00037	4425	3391	ALCOHOL DEHYDROGENASE (EC 1.1.1.1)
211	212	RXA01571	GR00438	1360	1959	ALCOHOL DEHYDROGENASE (EC 1.1.1.1)
213	214	RXA01572	GR00438	1928	2419	ALCOHOL DEHYDROGENASE (EC 1.1.1.1)
215	216	RXA01758	GR00498	3961	2945	ALCOHOL DEHYDROGENASE (EC 1.1.1.1)
217	218	RXA02539	GR00726	11676	10159	ALDEHYDE DEHYDROGENASE (EC
219	220	RXN03061	VV0034	108	437	ALDEHYDE DEHYDROGENASE (EC 1.2.1.3)
221	222	RXN03150	VV0155	10678	10055	ALDEHYDE DEHYDROGENASE (EC 1.2.1.3)
223	224	RXN01340	VV0033	3	860	ALDEHYDE DEHYDROGENASE (EC 1.2.1.3)
225	226	RXN01498	VV0008	1598	3160	ALDEHYDE DEHYDROGENASE (EC 1.2.1.3)
227	228	RXN02674	VV0315	15614	14163	ACETOLACTATE SYNTHASE LARGE SUBUNIT (EC 4.1.3.18)
229	230	RXN00868	VV0127	2230	320	ACETOLACTATE SYNTHASE LARGE SUBUNIT (EC 4.1.3.18)
231	232	RXN01143	VV0077	9372	8254	ACETOLACTATE SYNTHASE LARGE SUBUNIT (EC 4.1.3.18)
233	234	RXN01148	VV0284	243	935	ACETOLACTATE SYNTHASE SMALL SUBUNIT (EC 4.1.3.18)
235	236	RXN01144	VV0077	8237	7722	

Butanediol, diacetyl and acetoin formation

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
237	238	RXA02474	GR00715	6082	7309	(S,S)-butane-2,3-diol dehydrogenase (EC 1.1.1.76)
239	240	RXA02453	GR00710	6103	5351	ACETOIN(DIACETYL) REDUCTASE (EC 1.1.1.5)
241	242	RXS01758	VV0112	27383	28399	ALCOHOL DEHYDROGENASE (EC 1.1.1.1)

Table 1 (continued)

HMP-Cycle

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
243	244	RXA02737	GR00763	3312	1771	GLUCOSE-6-PHOSPHATE 1-DEHYDROGENASE (EC 1.1.1.49)
245	246	RXA02738	GR00763	4499	3420	TRANSALDOLASE (EC 2.2.1.2)
247	248	RXA02739	GR00763	6769	4670	TRANSKETOLASE (EC 2.2.1.1)
249	250	RXA00965	GR00270	1232	510	6-PHOSPHOGLUCONATE DEHYDROGENASE, DECARBOXYLATING (EC 1.1.1.44)
251	252	RXN00999	VV0106	2817	1366	6-PHOSPHOGLUCONATE DEHYDROGENASE, DECARBOXYLATING (EC 1.1.1.44)
253	254	F RXA00999	GR00263	3012	4448	6-PHOSPHOGLUCONATE DEHYDROGENASE, DECARBOXYLATING (EC 1.1.1.44)

Nucleotide sugar conversion

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
255	256	RXN02596	VV0098	48784	47582	UDP-GALACTOPYRANOSE MUTASE (EC 5.4.99.9)
257	258	F RXA02596	GR00742	1	489	UDP-GALACTOPYRANOSE MUTASE (EC 5.4.99.9)
259	260	F RXA02642	GR00749	5383	5860	UDP-GALACTOPYRANOSE MUTASE (EC 5.4.99.9)
261	262	RXA02572	GR00737	2	646	UDP-GLUCOSE 6-DEHYDROGENASE (EC 1.1.1.22)
263	264	RXA02485	GR00718	2345	3445	UDP-N-ACETYLENOLPYRUVOYLGLUCOSAMINE REDUCTASE (EC 1.1.1.158)
265	266	RXA01216	GR00352	2302	1202	UDP-N-ACETYLGLUCOSAMINE PYROPHOSPHORYLASE (EC 2.7.7.23)
267	268	RXA01259	GR00367	987	130	UTP--GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (EC 2.7.7.9)
269	270	RXA02028	GR00616	573	998	UTP--GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (EC 2.7.7.9)
271	272	RXA01262	GR00367	8351	7191	GDP-MANNOSE 6-DEHYDROGENASE (EC 1.1.1.132)
273	274	RXA01377	GR00400	3935	5020	MANNOSE-1-PHOSPHATE GUANYLTRANSFERASE (EC 2.7.7.13)
275	276	RXA02063	GR00626	3301	4527	GLUCOSE-1-PHOSPHATE ADENYLYLTRANSFERASE (EC 2.7.7.27)
277	278	RXN00014	VV0048	8848	9627	GLUCOSE-1-PHOSPHATE THYMIDYLYLTRANSFERASE (EC 2.7.7.24)
279	280	F RXA00014	GR00002	4448	5227	GLUCOSE-1-PHOSPHATE THYMIDYLYLTRANSFERASE (EC 2.7.7.24)
281	282	RXA01570	GR00438	427	1281	GLUCOSE-1-PHOSPHATE THYMIDYLYLTRANSFERASE (EC 2.7.7.24)
283	284	RXA02666	GR00753	7260	8493	D-RIBITOL-5-PHOSPHATE CYTIDYLYLTRANSFERASE (EC 2.7.7.40)
285	286	RXA00825	GR00222	222	1154	DTDP-GLUCOSE 4,6-DEHYDRATASE (EC 4.2.1.46)

Inositol and ribitol metabolism

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
287	288	RXA01887	GR00539	4219	3209	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
289	290	RXN00013	VV0048	7966	8838	MYO-INOSITOL-1(OR 4)-MONOPHOSPHATASE 1 (EC 3.1.3.25)
291	292	F RXA00013	GR00002	3566	4438	MYO-INOSITOL-1(OR 4)-MONOPHOSPHATASE 1 (EC 3.1.3.25)
293	294	RXA01099	GR00306	6328	5504	INOSITOL MONOPHOSPHATE PHOSPHATASE
295	296	RXN01332	VV0273	579	4	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
297	298	F RXA01332	GR00388	552	4	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
299	300	RXA01632	GR00454	2338	3342	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
301	302	RXA01633	GR00454	3380	4462	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
303	304	RXN01406	VV0278	2999	1977	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
305	306	RXN01630	VV0050	48113	47037	MYO-INOSITOL-1-PHOSPHATE SYNTHASE (EC 5.5.1.4)
307	308	RXN00528	VV0079	23406	22318	MYO-INOSITOL 2-DEHYDROGENASE (EC 1.1.1.18)
309	310	RXN03057	VV0028	7017	7688	GLUCOSE-FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
311	312	F RXA02902	GR10040	10277	10948	RIBITOL 2-DEHYDROGENASE (EC 1.1.1.56)
313	314	RXA00251	GR00038	931	224	

Utilization of sugars

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
315	316	RXN02654	VV0090	12206	13090	GLUCOSE 1-DEHYDROGENASE (EC 1.1.1.47)
317	318	F RXA02654	GR00752	7405	8289	GLUCOSE 1-DEHYDROGENASE II (EC 1.1.1.47)
319	320	RXN01049	VV0079	9833	11114	GLUCONOKINASE (EC 2.7.1.12)
321	322	F RXA01049	GR00296	1502	492	GLUCONOKINASE (EC 2.7.1.12)
323	324	F RXA01050	GR00296	1972	1499	GLUCONOKINASE (EC 2.7.1.12)
325	326	RXA00202	GR00032	1216	275	D-RIBOSE-BINDING PERIPLASMIC PROTEIN PRECURSOR
327	328	RXN00872	VV0127	6557	5604	FRUCTOKINASE (EC 2.7.1.4)
329	330	F RXA00872	GR00240	565	1086	FRUCTOKINASE (EC 2.7.1.4)
331	332	RXN00799	VV0009	58477	56834	PERIPLASMIC BETA-GLUCOSIDASE/BETA-XYLOSIDASE PRECURSOR (EC 3.2.1.21) (EC 3.2.1.37)
333	334	F RXA00799	GR00214	1	1584	PERIPLASMIC BETA-GLUCOSIDASE/BETA-XYLOSIDASE PRECURSOR (EC 3.2.1.21) (EC 3.2.1.37)
335	336	RXA00032	GR00003	12028	10520	MANNITOL 2-DEHYDROGENASE (EC 1.1.1.67)
337	338	RXA02528	GR00725	6880	7854	FRUCTOSE REPRESSOR
339	340	RXN00316	VV0006	7035	8180	Hypothetical Oxidoreductase (EC 1.1.1.-)
341	342	F RXA00309	GR00053	316	5	GLUCOSE--FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
343	344	RXN00310	VV0006	6616	7050	GLUCOSE--FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
345	346	F RXA00310	GR00053	735	301	GLUCOSE--FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
347	348	RXA00041	GR00007	1246	5	SUCROSE-6-PHOSPHATE HYDROLASE (EC 3.2.1.26)
349	350	RXA02026	GR00615	725	6	SUCROSE-6-PHOSPHATE HYDROLASE (EC 3.2.1.26)
351	352	RXA02061	GR00626	1842	349	SUCROSE-6-PHOSPHATE HYDROLASE (EC 3.2.1.26)

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig	NT Start	NT Stop	Function
353	354	RXN01369	VV0124	595	1776	MANNOSE-6-PHOSPHATE ISOMERASE (EC 5.3.1.8)
355	356	F RXA01369	GR00398	3	503	MANNOSE-6-PHOSPHATE ISOMERASE (EC 5.3.1.8)
357	358	F RXA01373	GR00399	595	1302	MANNOSE-6-PHOSPHATE ISOMERASE (EC 5.3.1.8)
359	360	RXA02611	GR00743	1	1752	1,4-ALPHA-GLUCAN BRANCHING ENZYME (EC 2.4.1.18)
361	362	RXA02612	GR00743	1793	3985	1,4-ALPHA-GLUCAN BRANCHING ENZYME (EC 2.4.1.18)
363	364	RXN01884	VV0184	1	1890	GLYCOGEN DEBRANCHING ENZYME (EC 2.4.1.25) (EC 3.2.1.33)
365	366	F RXA01884	GR00539	3	1475	GLYCOGEN DEBRANCHING ENZYME (EC 2.4.1.25) (EC 3.2.1.33)
367	368	RXA01111	GR00306	16981	17427	GLYCOGEN OPERON PROTEIN GLGX (EC 3.2.1.-)
369	370	RXN01550	VV0143	14749	16260	GLYCOGEN PHOSPHORYLASE (EC 2.4.1.1)
371	372	F RXA01550	GR00431	3	1346	GLYCOGEN PHOSPHORYLASE (EC 2.4.1.1)
373	374	RXN02100	VV0318	2	2326	GLYCOGEN PHOSPHORYLASE (EC 2.4.1.1)
375	376	F RXA02100	GR00631	3	920	GLYCOGEN PHOSPHORYLASE (EC 2.4.1.1)
377	378	F RXA02113	GR00633	2	1207	GLYCOGEN PHOSPHORYLASE (EC 2.4.1.1)
379	380	RXA02147	GR00639	15516	16532	ALPHA-AMYLASE (EC 3.2.1.1)
381	382	RXA01478	GR00422	10517	12352	GLUCOAMYLASE G1 AND G2 PRECURSOR (EC 3.2.1.3)
383	384	RXA01888	GR00539	4366	4923	GLUCOSE-RESISTANCE AMYLASE REGULATOR
385	386	RXN01927	VV0127	50623	49244	XYLULOSE KINASE (EC 2.7.1.17)
387	388	F RXA01927	GR00555	3	1118	XYLULOSE KINASE (EC 2.7.1.17)
389	390	RXA02729	GR00762	747	4	RIBOKINASE (EC 2.7.1.15)
391	392	RXA02797	GR00778	1739	2641	RIBOKINASE (EC 2.7.1.15)
393	394	RXA02730	GR00782	1768	731	RIBOSE OPERON REPRESSOR
395	396	RXA02551	GR00729	2193	2552	6-PHOSPHO-BETA-GLUCOSIDASE (EC 3.2.1.86)
397	398	RXA01325	GR00385	5676	5005	DEOXYRIBOSE-PHOSPHATE ALDOLASE (EC 4.1.2.4)
399	400	RXA00195	GR00030	543	1103	1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.-)
401	402	RXA00196	GR00030	1094	1708	1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.-)
403	404	RXN01562	VV0191	1230	3137	1-DEOXYXYLULOSE-5-PHOSPHATE SYNTHASE
405	406	F RXA01562	GR00436	2	1039	1-DEOXYXYLULOSE-5-PHOSPHATE SYNTHASE
407	408	F RXA01705	GR00480	971	1573	1-DEOXYXYLULOSE-5-PHOSPHATE SYNTHASE
409	410	RXN00879	VV0099	8763	6846	4-ALPHA-GLUCANOTRANSFERASE (EC 2.4.1.25)
411	412	F RXA00879	GR00242	5827	3828	4-ALPHA-GLUCANOTRANSFERASE (EC 2.4.1.25), amylomaltase
413	414	RXN00043	VV0119	3244	2081	N-ACETYLGLUCOSAMINE-6-PHOSPHATE DEACETYLASE (EC 3.5.1.25)
415	416	F RXA00043	GR00007	3244	2081	N-ACETYLGLUCOSAMINE-6-PHOSPHATE DEACETYLASE (EC 3.5.1.25)
417	418	RXN01752	VV0127	35265	33805	N-ACETYLGLUCOSAMINYLTRANSFERASE (EC 2.4.1.-)
419	420	F RXA01839	GR00520	1157	510	N-ACETYLGLUCOSAMINYLTRANSFERASE (EC 2.4.1.-)
421	422	RXA01859	GR00529	1473	547	N-ACETYLGLUCOSAMINYLTRANSFERASE (EC 2.4.1.-)
423	424	RXA00042	GR00007	2037	1279	GLUCOSAMINE-6-PHOSPHATE ISOMERASE (EC 5.3.1.10)
425	426	RXA01482	GR00422	17271	15397	GLUCOSAMINE--FRUCTOSE-6-PHOSPHATE AMINOTRANSFERASE (ISOMERIZING) (EC 2.6.1.16)
427	428	RXN03179	VV0336	2	667	URONATE ISOMERASE (EC 5.3.1.12)
429	430	F RXA02872	GR10013	675	4	URONATE ISOMERASE, Glucuronate isomerase (EC 5.3.1.12)
431	432	RXN03180	VV0337	672	163	URONATE ISOMERASE (EC 5.3.1.12)
433	434	F RXA02873	GR10014	672	163	URONATE ISOMERASE, Glucuronate isomerase (EC 5.3.1.12)
435	436	RXA02292	GR00662	1611	2285	GALACTOSIDE O-ACETYLTRANSFERASE (EC 2.3.1.18)
437	438	RXA02666	GR00753	7260	6493	D-RIBITOL-5-PHOSPHATE CYTIDYLYLTRANSFERASE (EC 2.7.7.40)
439	440	RXA00202	GR00032	1216	275	D-RIBOSE-BINDING PERIPLASMIC PROTEIN PRECURSOR
441	442	RXA02440	GR00709	5097	4258	D-RIBOSE-BINDING PERIPLASMIC PROTEIN PRECURSOR

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
443	444	RXN01569	VV0009	41086	42444	dTDP-4-DEHYDRORHAMNOSE REDUCTASE (EC 1.1.1.133)
445	446	F RXA01569	GR00438	2	427	DTDP-4-DEHYDRORHAMNOSE REDUCTASE (EC 1.1.1.133)
447	448	F RXA02055	GR00624	7122	8042	DTDP-4-DEHYDRORHAMNOSE REDUCTASE (EC 1.1.1.133)
449	450	RXA00825	GR00222	222	1154	DTDP-GLUCOSE 4,6-DEHYDRATASE (EC 4.2.1.46)
451	452	RXA02054	GR00624	6103	7119	DTDP-GLUCOSE 4,6-DEHYDRATASE (EC 4.2.1.46)
453	454	RXN00427	VV0112	7004	8219	dTDP-RHAMNOSYL TRANSFERASE RFBF (EC 2.-.-.-)
455	456	F RXA00427	GR00098	1591	2022	DTDP-RHAMNOSYL TRANSFERASE RFBF (EC 2.-.-.-)
457	458	RXA00327	GR00057	10263	9880	PROTEIN ARAJ
459	460	RXA00328	GR00057	11147	10656	PROTEIN ARAJ
461	462	RXA00329	GR00057	12390	11167	PROTEIN ARAJ
463	464	RXN01554	VV0135	28686	26545	GLUCAN ENDO-1,3-BETA-GLUCOSIDASE A1 PRECURSOR (EC 3.2.1.39)
465	466	RXN03015	VV0083	289	8	UDP-GLUCOSE 6-DEHYDROGENASE (EC 1.1.1.22)
467	468	RXN03056	VV0028	6258	6935	PUTATIVE HEXULOSE-6-PHOSPHATE ISOMERASE (EC 5.-.-.-)
469	470	RXN03030	VV0009	57006	56443	PERIPLASMIC BETA-GLUCOSIDASE/BETA-XYLOSIDASE PRECURSOR (EC 3.2.1.21) (EC 3.2.1.37)
471	472	RXN00401	VV0025	12427	11489	5-DEHYDRO-4-DEOXYGLUCARATE DEHYDRATASE (EC 4.2.1.41)
473	474	RXN02125	VV0102	23242	22442	ALDOSE REDUCTASE (EC 1.1.1.21)
475	476	RXN00200	VV0181	1679	5116	arabinosyl transferase subunit B (EC 2.4.2.-)
477	478	RXN01175	VV0017	39688	38303	PHOSPHO-2-DEHYDRO-3-DEOXYHEPTONATE ALDOLASE (EC 4.1.2.15)
479	480	RXN01376	VV0091	5610	4750	PUTATIVE GLYCOSYL TRANSFERASE WBIF
481	482	RXN01631	VV0050	47021	46143	PUTATIVE HEXULOSE-6-PHOSPHATE ISOMERASE (EC 5.-.-.-)
483	484	RXN01593	VV0229	13274	12408	NAGD PROTEIN
485	486	RXN00337	VV0197	20369	21418	GALACTOKINASE (EC 2.7.1.6)
487	488	RXS00584	VV0323	5516	6840	PHOSPHO-2-DEHYDRO-3-DEOXYHEPTONATE ALDOLASE (EC 4.1.2.15)
489	490	RXS02574				BETA-HEXOSAMINIDASE A PRECURSOR (EC 3.2.1.52)
491	492	RXS03215				GLUCOSE--FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
493	494	F RXA01915	GR00549	1	1008	GLUCOSE--FRUCTOSE OXIDOREDUCTASE PRECURSOR (EC 1.1.99.28)
495	496	RXS03224				CYCLOMALTODEXTRINASE (EC 3.2.1.54)
497	498	F RXA00038	GR00006	1417	260	CYCLOMALTODEXTRINASE (EC 3.2.1.54)
499	500	RXC00233				protein involved in sugar metabolism
501	502	RXC00236				Membrane Lipoprotein involved in sugar metabolism
503	504	RXC00271				Exported Protein involved in ribose metabolism
505	506	RXC00338				protein involved in sugar metabolism
507	508	RXC00362				Membrane Spanning Protein involved in metabolism of diols
509	510	RXC00412				Amino Acid ABC Transporter ATP-Binding Protein involved in sugar metabolism
511	512	RXC00526				ABC Transporter ATP-Binding Protein involved in sugar metabolism
513	514	RXC01004				Membrane Spanning Protein involved in sugar metabolism
515	516	RXC01017				Cytosolic Protein involved in sugar metabolism
517	518	RXC01021				Cytosolic Kinase involved in metabolism of sugars and thiamin
519	520	RXC01212				ABC Transporter ATP-Binding Protein involved in sugar metabolism
521	522	RXC01306				Membrane Spanning Protein involved in sugar metabolism
523	524	RXC01366				Cytosolic Protein involved in sugar metabolism
525	526	RXC01372				Cytosolic Protein involved in sugar metabolism

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
527	528	RXC01659				protein involved in sugar metabolism
529	530	RXC01663				protein involved in sugar metabolism
531	532	RXC01693				protein involved in sugar metabolism
533	534	RXC01703				Cytosolic Protein involved in sugar metabolism
535	536	RXC02254				Membrane Associated Protein involved in sugar metabolism
537	538	RXC02255				Cytosolic Protein involved in sugar metabolism
539	540	RXC02435				protein involved in sugar metabolism
541	542	F RXA02435	GR00709	825	268	Uncharacterized protein involved in glycerol metabolism (homolog of Drosophila rhomboid)
543	544	RXC03216				protein involved in sugar metabolism

TCA-cycle

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
545	546	RXA02175	GR00641	10710	9418	CITRATE SYNTHASE (EC 4.1.3.7)
547	548	RXA02621	GR00746	2647	1829	CITRATE LYASE BETA CHAIN (EC 4.1.3.6)
549	550	RXN00519	VV0144	5585	3372	ISOCITRATE DEHYDROGENASE (NADP) (EC 1.1.1.42)
551	552	F RXA00521	GR00133	2	1060	ISOCITRATE DEHYDROGENASE [NADP] (EC 1.1.1.42)
553	554	RXN02209	VV0304	1	1671	ACONITATE HYDRATASE (EC 4.2.1.3)
555	556	F RXA02209	GR00648	3	1661	ACONITATE HYDRATASE (EC 4.2.1.3)
557	558	RXN02213	VV0305	1378	2151	ACONITATE HYDRATASE (EC 4.2.1.3)
559	560	F RXA02213	GR00649	1330	2046	ACONITATE HYDRATASE (EC 4.2.1.3)
561	562	RXA02056	GR00625	3	2870	2-OXOGLUTARATE DEHYDROGENASE E1 COMPONENT (EC 1.2.4.2)
563	564	RXA01745	GR00495	2	1495	DIHYDROLIPOAMIDE SUCCINYLTRANSFERASE COMPONENT (E2) OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX (EC 2.3.1.61)
565	566	RXA00782	GR00206	3984	3103	SUCCINYL-COA SYNTHETASE ALPHA CHAIN (EC 6.2.1.5)
567	568	RXA00783	GR00206	5280	4009	SUCCINYL-COA SYNTHETASE BETA CHAIN (EC 6.2.1.5)
569	570	RXN01695	VV0139	11307	12806	L-MALATE DEHYDROGENASE (ACCEPTOR) (EC 1.1.99.16)
571	572	F RXA01615	GR00449	8608	9546	L-MALATE DEHYDROGENASE (ACCEPTOR) (EC 1.1.99.16)
573	574	F RXA01695	GR00474	4388	4179	L-MALATE DEHYDROGENASE (ACCEPTOR) (EC 1.1.99.16)
575	576	RXA00290	GR00046	4893	5855	MALIC ENZYME (EC 1.1.1.39)
577	578	RXN01048	VV0079	12539	11316	MALIC ENZYME (EC 1.1.1.39)
579	580	F RXA01048	GR00296	3	290	MALIC ENZYME (EC 1.1.1.39)
581	582	F RXA00290	GR00046	4893	5855	MALIC ENZYME (EC 1.1.1.39)
583	584	RXN03101	VV0068	2	583	DIHYDROLIPOAMIDE SUCCINYLTRANSFERASE COMPONENT (E2) OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX (EC 2.3.1.61)
585	586	RXN02046	VV0025	15056	14640	DIHYDROLIPOAMIDE SUCCINYLTRANSFERASE COMPONENT OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX (EC 2.3.1.61)
587	588	RXN00389	VV0025	11481	9922	oxoglutarate semialdehyde dehydrogenase (EC 1.2.1.-)

Table 1 (continued)

Glyoxylate bypass

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
589	590	RXN02399	VV0176	19708	18365	ISOCITRATE LYASE (EC 4.1.3.1)
591	592	F RXA02399	GR00699	478	1773	ISOCITRATE LYASE (EC 4.1.3.1)
593	594	RXN02404	VV0176	20259	22475	MALATE SYNTHASE (EC 4.1.3.2)
595	596	F RXA02404	GR00700	3798	1663	MALATE SYNTHASE (EC 4.1.3.2)
597	598	RXA01089	GR00304	3209	3958	GLYOXYLATE-INDUCED PROTEIN
599	600	RXA01888	GR00539	3203	2430	GLYOXYLATE-INDUCED PROTEIN

Methylcitrate-pathway

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
600	602	RXN03117	VV0092	3087	1576	2-methylisocitrate synthase (EC 5.3.3.-)
601	604	F RXA00406	GR00090	978	4	2-methylisocitrate synthase (EC 5.3.3.-)
603	606	F RXA00514	GR00130	1983	1576	2-methylisocitrate synthase (EC 5.3.3.-)
605	608	RXA00512	GR00130	621	4	2-methylcitrate synthase (EC 4.1.3.31)
607	610	RXA00518	GR00131	3069	2773	2-methylcitrate synthase (EC 4.1.3.31)
609	612	RXA01077	GR00300	4647	6017	2-methylisocitrate synthase (EC 5.3.3.-)
611	614	RXN03144	VV0141	2	901	2-methylisocitrate synthase (EC 5.3.3.-)
613	616	F RXA02322	GR00668	415	5	2-methylisocitrate synthase (EC 5.3.3.-)
615	618	RXA02329	GR00669	607	5	2-methylisocitrate synthase (EC 5.3.3.-)
617	620	RXA02332	GR00671	1906	764	2-methylcitrate synthase (EC 4.1.3.31)
619	622	RXN02333	VV0141	901	1815	methylisocitrate lyase (EC 4.1.3.30)
621	624	F RXA02333	GR00671	2120	1902	methylisocitrate lyase (EC 4.1.3.30)
623	626	RXA00030	GR00003	9590	9979	LACTOYLGLUTATHIONE LYASE (EC 4.4.1.5)

Methyl-Malonyl-CoA-Mutases

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
625	628	RXN00148	VV0167	9849	12059	METHYLMALONYL-COA MUTASE ALPHA-SUBUNIT (EC 5.4.99.2)
627	630	F RXA00148	GR00023	2002	5	METHYLMALONYL-COA MUTASE ALPHA-SUBUNIT (EC 5.4.99.2)
629	632	RXA00149	GR00023	3856	2009	METHYLMALONYL-COA MUTASE BETA-SUBUNIT (EC 5.4.99.2)

Table 1 (continued)

Others

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
631	634	RXN00317	VV0197	26879	27532	PHOSPHOGLYCOLATE PHOSPHATASE (EC 3.1.3.18)
635	636	F RXA00317	GR00055	344	6	PHOSPHOGLYCOLATE PHOSPHATASE (EC 3.1.3.18)
637	638	RXA02196	GR00645	3956	3264	PHOSPHOGLYCOLATE PHOSPHATASE (EC 3.1.3.18)
639	640	RXN02461	VV0124	14236	14643	PHOSPHOGLYCOLATE PHOSPHATASE (EC 3.1.3.18)

Redox Chain

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
641	642	RXN01744	VV0174	2350	812	CYTOCHROME D UBIQUINOL OXIDASE SUBUNIT I (EC 1.10.3.-)
643	644	F RXA00055	GR00008	11753	11890	CYTOCHROME D UBIQUINOL OXIDASE SUBUNIT I (EC 1.10.3.-)
645	646	F RXA01744	GR00494	2113	812	CYTOCHROME D UBIQUINOL OXIDASE SUBUNIT I (EC 1.10.3.-)
647	648	RXA00379	GR00082	212	6	CYTOCHROME C-TYPE BIOGENESIS PROTEIN CCDA
649	650	RXA00385	GR00083	773	435	CYTOCHROME C-TYPE BIOGENESIS PROTEIN CCDA
651	652	RXA01743	GR00494	806	6	CYTOCHROME D UBIQUINOL OXIDASE SUBUNIT II (EC 1.10.3.-)
653	654	RXN02480	VV0084	31222	29567	CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1)
655	656	F RXA01919	GR00550	288	4	CYTOCHROME C OXIDASE SUBUNIT I (EC 1.9.3.1)
657	658	F RXA02480	GR00717	1449	601	CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1)
659	660	F RXA02481	GR00717	1945	1334	CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1)
661	662	RXA02140	GR00639	7339	8415	CYTOCHROME C OXIDASE POLYPEPTIDE II (EC 1.9.3.1)
663	664	RXA02142	GR00639	9413	10063	CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1)
665	666	RXA02144	GR00639	11025	12248	RIESKE IRON-SULFUR PROTEIN
667	668	RXA02740	GR00763	7613	8542	PROBABLE CYTOCHROME C OXIDASE ASSEMBLY FACTOR
669	670	RXA02743	GR00763	13534	12497	CYTOCHROME AA3 CONTROLLING PROTEIN
671	672	RXA01227	GR00355	1199	1519	FERREDOXIN
673	674	RXA01865	GR00532	436	122	FERREDOXIN
675	676	RXA00680	GR00179	2632	2315	FERREDOXIN VI
677	678	RXA00679	GR00179	2302	1037	FERREDOXIN--NAD(+) REDUCTASE (EC 1.18.1.3)
679	680	RXA00224	GR00032	24965	24015	ELECTRON TRANSFER FLAVOPROTEIN ALPHA-SUBUNIT
681	682	RXA00225	GR00032	25783	24996	ELECTRON TRANSFER FLAVOPROTEIN BETA-SUBUNIT
683	684	RXN00606	VV0192	11299	9026	NADH DEHYDROGENASE I CHAIN L (EC 1.6.5.3)
685	686	F RXA00606	GR00160	121	1869	NADH DEHYDROGENASE I CHAIN L (EC 1.6.5.3)
687	688	RXN00595	VV0192	8842	7113	NADH DEHYDROGENASE I CHAIN M (EC 1.6.5.3)
689	690	F RXA00608	GR00160	2253	3017	NADH DEHYDROGENASE I CHAIN M (EC 1.6.5.3)
691	692	RXA00913	GR00249	3	2120	NADH DEHYDROGENASE I CHAIN L (EC 1.6.5.3)
693	694	RXA00909	GR00247	2552	3406	NADH DEHYDROGENASE I CHAIN L (EC 1.6.5.3)
695	696	RXA00700	GR00182	846	43	NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 2
697	698	RXN00483	VV0088	44824	46287	NADH-UBIQUINONE OXIDOREDUCTASE 39 KD SUBUNIT PRECURSOR (EC 1.6.5.3) (EC 1.6.99.3)

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
699	700	F RXA00483	GR00119	19108	20569	NADH-UBIQUINONE OXIDOREDUCTASE 39 KD SUBUNIT PRECURSOR (EC 1.6.5.3) (EC 1.6.99.3)
701	702	RXA01534	GR00427	1035	547	NADH-DEPENDENT FMN OXYDOREDUCTASE
703	704	RXA00288	GR00046	2646	1636	QUINONE OXIDOREDUCTASE (EC 1.6.5.5)
705	706	RXA02741	GR00763	9585	8620	QUINONE OXIDOREDUCTASE (EC 1.6.5.5)
707	708	RXN02560	VV0101	9922	10768	NADPH-FLAVIN OXIDOREDUCTASE (EC 1.6.99.-)
709	710	F RXA02560	GR00731	6339	7160	NADPH-FLAVIN OXIDOREDUCTASE (EC 1.6.99.-)
711	712	RXA01311	GR00380	1611	865	SUCCINATE DEHYDROGENASE IRON-SULFUR PROTEIN (EC 1.3.99.1)
713	714	RXN03014	VV0058	1273	368	NADH DEHYDROGENASE I CHAIN M (EC 1.6.5.3)
715	716	F RXA00910	GR00246	3	1259	Hydrogenase subunits
717	718	RXN01695	VV0117	955	5	NADH DEHYDROGENASE (EC 1.6.99.3)
719	720	F RXA01895	GR00543	2	817	DEHYDROGENASE
721	722	RXA00703	GR00183	2556	271	FORMATE DEHYDROGENASE ALPHA CHAIN (EC 1.2.1.2)
723	724	RXN00705	VV0005	8111	5197	FDHD PROTEIN
725	726	F RXA00705	GR00184	1291	407	FDHD PROTEIN
727	728	RXN00388	VV0025	2081	3091	CYTOCHROME C BIOGENESIS PROTEIN CCSA
729	730	F RXA00388	GR00085	969	667	essential protein similar to cytochrome c
731	732	F RXA00386	GR00084	514	5	RESC PROTEIN, essential protein similar to cytochrome c biogenesis protein
733	734	RXA00945	GR00259	1876	2847	putative cytochrome oxidase
735	736	RXN02558	VV0101	5602	6759	FLAVOHEMOPROTEIN / DIHYDROPTERIDINE REDUCTASE (EC 1.6.99.7)
737	738	F RXA02558	GR00731	2019	3176	FLAVOHEMOPROTEIN
739	740	RXA01392	GR00408	2297	3373	GLUTATHIONE S-TRANSFERASE (EC 2.5.1.18)
741	742	RXA00800	GR00214	2031	3134	GLUTATHIONE-DEPENDENT FORMALDEHYDE DEHYDROGENASE (EC 1.2.1.1)
743	744	RXA02143	GR00639	10138	11025	QCRC PROTEIN, menaquinol:cytochrome c oxidoreductase
745	746	RXN03096	VV0058	405	4	NADH DEHYDROGENASE I CHAIN M (EC 1.6.5.3)
747	748	RXN02036	VV0176	32683	33063	NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 4 (EC 1.6.5.3)
749	750	RXN02765	VV0317	3552	2794	Hypothetical Oxidorductase
751	752	RXN02206	VV0302	1784	849	Hypothetical Oxidoreductase
753	754	RXN02554	VV0101	4633	4010	Hypothetical Oxidoreductase (EC 1.1.1.-)

ATP-Synthase

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
755	756	RXN01204	VV0121	1270	461	ATP SYNTHASE A CHAIN (EC 3.6.1.34)
757	758	F RXA01204	GR00345	394	1155	ATP SYNTHASE A CHAIN (EC 3.6.1.34)
759	760	RXA01201	GR00344	675	2315	ATP SYNTHASE ALPHA CHAIN (EC 3.6.1.34)
761	762	RXN01193	VV0175	5280	3832	ATP SYNTHASE BETA CHAIN (EC 3.6.1.34)
763	764	F RXA01193	GR00343	15	755	ATP SYNTHASE BETA CHAIN (EC 3.6.1.34)
765	766	F RXA01203	GR00344	3355	3993	ATP SYNTHASE BETA CHAIN (EC 3.6.1.34)

Table 1 (continued)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
767	768	RXN02821	VV0121	324	85	ATP SYNTHASE C CHAIN (EC 3.6.1.34)
769	770	F RXA02821	GR00802	139	318	ATP SYNTHASE C CHAIN (EC 3.6.1.34)
771	772	RXA01200	GR00344	2	610	ATP SYNTHASE DELTA CHAIN (EC 3.6.1.34)
773	774	RXA01194	GR00343	770	1141	ATP SYNTHASE EPSILON CHAIN (EC 3.6.1.34)
775	776	RXA01202	GR00344	2375	3349	ATP SYNTHASE GAMMA CHAIN (EC 3.6.1.34)
777	778	RXN02434	VV0090	4923	3274	ATP-BINDING PROTEIN

Cytochrome metabolism

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
779	780	RXN00684	VV0005	29864	28581	CYTOCHROME P450 116 (EC 1.14.-.-)
781	782	RXN00387	VV0025	1150	2004	Hypothetical Cytochrome c Biogenesis Protein

TABLE 2 – Excluded Genes

GenBank™ Accession No.	Gene Name	Gene Function	Reference
A09073	ppg	Phosphoenol pyruvate carboxylase	Bachmann, B. et al. "DNA fragment coding for phosphoenolpyruvat corboxylase, recombinant DNA carrying said fragment, strains carrying the recombinant DNA and method for producing L-aminino acids using said strains," Patent: EP 0358940-A 3 03/21/90
A45579, A45581, A45583, A45585 A45587		Threonine dehydratase	Moeckel, B. et al. "Production of L-isoleucine by means of recombinant micro-organisms with deregulated threonine dehydratase," Patent: WO 9519442-A 5 07/20/95
AB003132	murC; ftsQ; ftsZ		Kobayashi, M. et al. "Cloning, sequencing, and characterization of the ftsZ gene from coryneform bacteria," *Biochem. Biophys. Res. Commun.*, 236(2):383-388 (1997)
AB015023	murC; ftsQ		Wachi, M. et al. "A murC gene from Coryneform bacteria," *Appl. Microbiol. Biotechnol.*, 51(2):223-228 (1999)
AB018530	dtsR		Kimura, E. et al. "Molecular cloning of a novel gene, dtsR, which rescues the detergent sensitivity of a mutant derived from *Brevibacterium lactofermentum*," *Biosci. Biotechnol. Biochem.*, 60(10):1565-1570 (1996)
AB018531	dtsR1; dtsR2		
AB020624	murI	D-glutamate racemase	
AB023377	tkt	transketolase	
AB024708	gltB; gltD	Glutamine 2-oxoglutarate aminotransferase large and small subunits	
AB025424	acn	aconitase	
AB027714	rep	Replication protein	
AB027715	rep; aad	Replication protein; aminoglycoside adenyltransferase	
AF005242	argC	N-acetylglutamate-5-semialdehyde dehydrogenase	
AF005635	glnA	Glutamine synthetase	
AF030405	hisF	cyclase	
AF030520	argG	Argininosuccinate synthetase	
AF031518	argF	Ornithine carbamolytransferase	
AF036932	aroD	3-dehydroquinate dehydratase	
AF038548	pyc	Pyruvate carboxylase	

Table 2 (continued)

AF038651	dciAE; apt; rel	Dipeptide-binding protein; adenine phosphoribosyltransferase; GTP pyrophosphokinase	Wehmeier, L. et al. "The role of the Corynebacterium glutamicum rel gene in (p)ppGpp metabolism," *Microbiology*, 144:1853-1862 (1998)
AF041436	argR	Arginine repressor	
AF045998	impA	Inositol monophosphate phosphatase	
AF048764	argH	Argininosuccinate lyase	
AF049897	argC; argJ; argB; argD; argF; argR; argG; argH	N-acetylglutamylphosphate reductase; ornithine acetyltransferase; N-acetylglutamate kinase; acetylornithine transminase; ornithine carbamoyltransferase; arginine repressor; argininosuccinate synthase; argininosuccinate lyase	
AF050109	inhA	Enoyl-acyl carrier protein reductase	
AF050166	hisG	ATP phosphoribosyltransferase	
AF051846	hisA	Phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazolecarboxamide isomerase	
AF052652	metA	Homoserine O-acetyltransferase	Park, S. et al. "Isolation and analysis of metA, a methionine biosynthetic gene encoding homoserine acetyltransferase in Corynebacterium glutamicum," *Mol. Cells.*, 8(3):286-294 (1998)
AF053071	aroB	Dehydroquinate synthetase	
AF060558	hisH	Glutamine amidotransferase	
AF086704	hisE	Phosphoribosyl-ATP-pyrophosphohydrolase	
AF114233	aroA	5-enolpyruvylshikimate 3-phosphate synthase	
AF116184	panD	L-aspartate-alpha-decarboxylase precursor	Dusch, N. et al. "Expression of the Corynebacterium glutamicum panD gene encoding L-aspartate-alpha-decarboxylase leads to pantothenate overproduction in Escherichia coli," *Appl. Environ. Microbiol.*, 65(4)1530-1539 (1999)
AF124518	aroD; aroE	3-dehydroquinase; shiklmate dehydrogenase	
AF124600	aroC; aroK; aroB; pepQ	Chorismate synthase; shikimate kinase; 3-dehydroquinate synthase; putative cytoplasmic peptidase	
AF145897	inhA		
AF145898	inhA		

Table 2 (continued)

AJ001436	ectP	Transport of ectoine, glycine betaine, proline	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes: Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP," *J. Bacteriol.*, 180(22):6005-6012 (1998)
AJ004934	dapD	Tetrahydrodipicolinate succinylase (incomplete)	Wehrmann, A. et al. "Different modes of diaminopimelate synthesis and their role in cell wall integrity: A study with Corynebacterium glutamicum," *J. Bacteriol.*, 180(12):3159-3165 (1998)
AJ007732	ppc; secG; amt; ocd; soxA	Phosphoenolpyruvate-carboxylase; ?; high affinity ammonium uptake protein; putative ornithine-cyclodecarboxylase; sarcosine oxidase	
AJ010319	ftsY, glnB, glnD; srp; amtP	Involved in cell division; PII protein; uridylyltransferase (uridylyl-removing enzmye); signal recognition particle; low affinity ammonium uptake protein	Jakoby, M. et al. "Nitrogen regulation in Corynebacterium glutamicum; Isolation of genes involved in biochemical characterization of corresponding proteins," *FEMS Microbiol.*, 173(2):303-310 (1999)
AJ132968	cat	Chloramphenicol aceteyl transferase	
AJ224946	mqo	L-malate: quinone oxidoreductase	Molenaar, D. et al. "Biochemical and genetic characterization of the membrane-associated malate dehydrogenase (acceptor) from Corynebacterium glutamicum," *Eur. J. Biochem.*, 254(2):395-403 (1998)
AJ238250	ndh	NADH dehydrogenase	
AJ238703	porA	Porin	Lichtinger, T. et al. "Biochemical and biophysical characterization of the cell wall porin of Corynebacterium glutamicum: The channel is formed by a low molecular mass polypeptide," *Biochemistry*, 37(43):15024-15032 (1998)
D17429		Transposable element IS31831	Vertes et al."Isolation and characterization of IS31831, a transposable element from Corynebacterium glutamicum," *Mol. Microbiol.*, 11(4):739-746 (1994)
D84102	odhA	2-oxoglutarate dehydrogenase	Usuda, Y. et al. "Molecular cloning of the Corynebacterium glutamicum (Brevibacterium lactofermentum AJ12036) odhA gene encoding a novel type of 2-oxoglutarate dehydrogenase," *Microbiology*, 142:3347-3354 (1996)
E01358	hdh; hk	Homoserine dehydrogenase; homoserine kinase	Katsumata, R. et al. "Production of L-thereonine and L-isoleucine," Patent: JP 1987232392-A 1 10/12/87
E01359		Upstream of the start codon of homoserine kinase gene	Katsumata, R. et al. "Production of L-thereonine and L-isoleucine," Patent: JP 1987232392-A 2 10/12/87
E01375		Tryptophan operon	
E01376	trpL; trpE	Leader peptide; anthranilate synthase	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87

Table 2 (continued)			
E01377		Promoter and operator regions of tryptophan operon	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87
E03937		Biotin-synthase	Hatakeyama, K. et al. "DNA fragment containing gene capable of coding biotin synthetase and its utilization," Patent: JP 1992278088-A 1 10/02/92
E04040		Diamino pelargonic acid aminotransferase	Kohama, K. et al. "Gene coding diaminopelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04041		Desthiobiotinsynthetase	Kohama, K. et al. "Gene coding diaminopelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04307		Flavum aspartase	Kurusu, Y. et al. "Gene DNA coding aspartase and utilization thereof," Patent: JP 1993030977-A 1 02/09/93
E04376		Isocitric acid lyase	Katsumata, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993056782-A 3 03/09/93
E04377		Isocitric acid lyase N-terminal fragment	Katsumata, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993056782-A 3 03/09/93
E04484		Prephenate dehydratase	Sotouchi, N. et al. "Production of L-phenylalanine by fermentation," Patent: JP 1993076352-A 2 03/30/93
E05108		Aspartokinase	Fugono, N. et al. "Gene DNA coding Aspartokinase and its use," Patent: JP 1993184366-A 1 07/27/93
E05112		Dihydro-dipichorinate synthetase	Hatakeyama, K. et al. "Gene DNA coding dihydrodipicolinic acid synthetase and its use," Patent: JP 1993184371-A 1 07/27/93
E05776		Diaminopimelic acid dehydrogenase	Kobayashi, M. et al. "Gene DNA coding Diaminopimelic acid dehydrogenase and its use," Patent: JP 1993284970-A 1 11/02/93
E05779		Threonine synthase	Kohama, K. et al. "Gene DNA coding threonine synthase and its use," Patent: JP 1993284972-A 1 11/02/93
E06110		Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent: JP 1993344881-A 1 12/27/93
E06111		Mutated Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent: JP 1993344881-A 1 12/27/93
E06146		Acetohydroxy acid synthetase	Inui, M. et al. "Gene capable of coding Acetohydroxy acid synthetase and its use," Patent: JP 1993344893-A 1 12/27/93
E06825		Aspartokinase	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94
E06826		Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94

Table 2 (continued)

E06827		Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94
E07701	secY		Honno, N. et al. "Gene DNA participating in integration of membraneous protein to membrane," Patent: JP 1994169780-A 1 06/21/94
E08177		Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent: JP 1994261766-A 1 09/20/94
E08178, E08179, E08180, E08181, E08182		Feedback inhibition-released Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent: JP 1994261766-A 1 09/20/94
E08232		Acetohydroxy-acid isomeroreductase	Inui, M. et al. "Gene DNA coding acetohydroxy acid isomeroreductase," Patent: JP 1994277067-A 1 10/04/94
E08234	secE		Asai, Y. et al. "Gene DNA coding for translocation machinery of protein," Patent: JP 1994277073-A 1 10/04/94
E08643		FT aminotransferase and desthiobiotin synthetase promoter region	Hatakeyama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031476-A 1 02/03/95
E08646		Biotin synthetase	Hatakeyama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031476-A 1 02/03/95
E08649		Aspartase	Kohama, K. et al "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031478-A 1 02/03/95
E08900		Dihydrodipicolinate reductase	Madori, M. et al. "DNA fragment containing gene coding Dihydrodipicolinate acid reductase and utilization thereof," Patent: JP 1995075578-A 1 03/20/95
E08901		Diaminopimelic acid decarboxylase	Madori, M. et al. "DNA fragment containing gene coding Diaminopimelic acid decarboxylase and utilization thereof," Patent: JP 1995075579-A 1 03/20/95
E12594		Serine hydroxymethyltransferase	Hatakeyama, K. et al. "Production of L-trypophan," Patent: JP 1997028391-A 1 02/04/97
E12760, E12759, E12758		transposase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12764		Arginyl-tRNA synthetase; diaminopimelic acid decarboxylase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12767		Dihydrodipicolinic acid synthetase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12770		aspartokinase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12773		Dihydrodipicolinic acid reductase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97

Table 2 (continued)

E13655		Glucose-6-phosphate dehydrogenase	Hatakeyama, K. et al. "Glucose-6-phosphate dehydrogenase and DNA capable of coding the same," Patent: JP 1997224661-A 1 09/02/97
L01508	IlvA	Threonine dehydratase	Moeckel, B. et al. "Functional and structural analysis of the threonine dehydratase of Corynebacterium glutamicum," *J. Bacteriol.*, 174:8065-8072 (1992)
L07603	EC 4.2.1.15	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase	Chen, C. et al. "The cloning and nucleotide sequence of Corynebacterium glutamicum 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase gene," *FEMS Microbiol. Lett.*, 107:223-230 (1993)
L09232	IlvB; ilvN; ilvC	Acetohydroxy acid synthase large subunit; Acetohydroxy acid synthase small subunit; Acetohydroxy acid isomeroreductase	Keilhauer, C. et al. "Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon," *J. Bacteriol.*, 175(17):5595-5603 (1993)
L18874	PtsM	Phosphoenolpyruvate sugar phosphotransferase	Fouet, A et al. "Bacillus subtilis sucrose-specific enzyme II of the phosphotransferase system: expression in Escherichia coli and homology to enzymes II from enteric bacteria," *PNAS USA*, 84(24):8773-8777 (1987); Lee, J.K. et al. "Nucleotide sequence of the gene encoding the Corynebacterium glutamicum mannose enzyme II and analyses of the deduced protein sequence," *FEMS Microbiol. Lett.*, 119(1-2):137-145 (1994)
L27123	aceB	Malate synthase	Lee, H-S. et al. "Molecular characterization of aceB, a gene encoding malate synthase in Corynebacterium glutamicum," *J. Microbiol. Biotechnol.*, 4(4):256-263 (1994)
L27126		Pyruvate kinase	Jetten, M. S. et al. "Structural and functional analysis of pyruvate kinase from Corynebacterium glutamicum," *Appl. Environ. Microbiol.*, 60(7):2501-2507 (1994)
L28760	aceA	Isocitrate lyase	
L35906	dtxr	Diphtheria toxin repressor	Oguiza, J.A. et al. "Molecular cloning, DNA sequence analysis, and characterization of the Corynebacterium diphtheriae dtxR from Brevibacterium lactofermentum," *J. Bacteriol.*, 177(2):465-467 (1995)
M13774		Prephenate dehydratase	Follettie, M.T. et al. "Molecular cloning and nucleotide sequence of the Corynebacterium glutamicum pheA gene," *J. Bacteriol.*, 167:695-702 (1986)
M16175	5S rRNA		Park, Y-H. et al. "Phylogenetic analysis of the coryneform bacteria by 56 rRNA sequences," *J. Bacteriol.*, 169:1801-1806 (1987)
M16663	trpE	Anthranilate synthase, 5' end	Sano, K. et al. "Structure and function of the trp operon control regions of Brevibacterium lactofermentum, a glutamic-acid-producing bacterium," *Gene*, 52:191-200 (1987)
M16664	trpA	Tryptophan synthase, 3'end	Sano, K. et al. "Structure and function of the trp operon control regions of Brevibacterium lactofermentum, a glutamic-acid-producing bacterium," *Gene*, 52:191-200 (1987)

Table 2 (continued)

M25819		Phosphoenolpyruvate carboxylase	O'Regan, M. et al. "Cloning and nucleotide sequence of the Phosphoenolpyruvate carboxylase-coding gene of Corynebacterium glutamicum ATCC13032," *Gene*, 77(2):237-251 (1989)
M85106		23S rRNA gene insertion sequence	Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," *J. Gen. Microbiol.*, 138:1167-1175 (1992)
M85107, M85108		23S rRNA gene insertion sequence	Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," *J. Gen. Microbiol.*, 138:1167-1175 (1992)
M89931	aecD; brnQ; yhbw	Beta C-S lyase; branched-chain amino acid uptake carrier; hypothetical protein yhbw	Rossol, I. et al. "The Corynebacterium glutamicum aecD gene encodes a C-S lyase with alpha, beta-elimination activity that degrades aminoethylcysteine," *J. Bacteriol.*, 174(9):2968-2977 (1992); Tauch, A. et al. "Isoleucine uptake in Corynebacterium glutamicum ATCC 13032 is directed by the brnQ gene product," *Arch. Microbiol.*, 169(4):303-312 (1998)
S59299	trp	Leader gene (promoter)	Herry, D.M. et al. "Cloning of the trp gene cluster from a tryptophan-hyperproducing strain of Corynebacterium glutamicum: identification of a mutation in the trp leader sequence," *Appl. Environ. Microbiol.*, 59(3):791-799 (1993)
U11545	trpD	Anthranilate phosphoribosyltransferase	O'Gara, J.P. and Dunican, L.K. (1994) Complete nucleotide sequence of the Corynebacterium glutamicum ATCC 21850 tpD gene." Thesis, Microbiology Department, University College Galway, Ireland.
U13922	cglIM; cglIR; clgIIR	Putative type II 5-cytosoine methyltransferase; putative type II restriction endonuclease; putative type I or type III restriction endonuclease	Schafer, A. et al. "Cloning and characterization of a DNA region encoding a stress-sensitive restriction system from Corynebacterium glutamicum ATCC 13032 and analysis of its role in intergeneric conjugation with Escherichia coli," *J. Bacteriol.*, 176(23):7309-7319 (1994); Schafer, A. et al. "The Corynebacterium glutamicum cglIM gene encoding a 5-cytosine in an McrBC-deficient Escherichia coli strain," *Gene*, 203(2):95-101 (1997)
U14965	recA		
U31224	ppx		Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)
U31225	proC	L-proline: NADP+ 5-oxidoreductase	Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)
U31230	obg; proB; unkdh	?;gamma glutamyl kinase;similar to D-isomer specific 2-hydroxyacid dehydrogenases	Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)

Table 2 (continued)

U31281	bioB	Biotin synthase	Serebriiskii, I.G., "Two new members of the bio B superfamily: Cloning, sequencing and expression of bio B genes of Methylobacillus flagellatum and Corynebacterium glutamicum," *Gene*, 175:15-22 (1996)
U35023	thtR; accBC	Thiosulfate sulfurtransferase; acyl CoA carboxylase	Jager, W. et al. "A Corynebacterium glutamicum gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins," *Arch. Microbiol.*, 166(2);76-82 (1996)
U43535	cmr	Multidrug resistance protein	Jager, W. et al. "A Corynebacterium glutamicum gene conferring multidrug resistance in the heterologous host Escherichia coli," *J. Bacteriol.*, 179(7):2449-2451 (1997)
U43536	clpB	Heat shock ATP-binding protein	
U53587	aphA-3	3"5"-aminoglycoside phosphotransferase	
U89648		Corynebacterium glutamicum unidentified sequence involved in histidine biosynthesis, partial sequence	
X04960	trpA; trpB; trpC; trpD; trpE; trpG; trpL	Tryptophan operon	Matsui, K. et al. "Complete nucleotide and deduced amino acid sequences of the Brevibacterium lactofermentum tryptophan operon," *Nucleic Acids Res.*, 14(24):10113-10114 (1986)
X07563	lys A	DAP decarboxylase (meso-diaminopimelate decarboxylase, EC 4.1.1.20)	Yeh, P. et al. "Nucleic sequence of the lysA gene of Corynebacterium glutamicum and possible mechanisms for modulation of its expression," *Mol. Gen. Genet.*, 212(1):112-119 (1988)
X14234	EC 4.1.1.31	Phosphoenolpyruvate carboxylase	Eikmanns, B.J. et al. "The Phosphoenolpyruvate carboxylase gene of Corynebacterium glutamicum: Molecular cloning, nucleotide sequence, and expression," *Mol. Gen. Genet.*, 218(2):330-339 (1989); Lepiniec, L. et al. "Sorghum Phosphoenolpyruvate carboxylase gene family: structure, function and molecular evolution," *Plant. Mol. Biol.*, 21 (3):487-502 (1993)
X17313	fda	Fructose-bisphosphate aldolase	Von der Osten, C.H. et al. "Molecular cloning, nucleotide sequence and fine-structural analysis of the Corynebacterium glutamicum fda gene: structural comparison of C. glutamicum fructose-1, 6-biphosphate aldolase to class I and class II aldolases," *Mol. Microbiol.*,
X53993	dapA	L-2, 3-dihydrodipicolinate synthetase (EC 4.2.1.52)	Bonnassie, S. et al. "Nucleic sequence of the dapA gene from Corynebacterium glutamicum," *Nucleic Acids Res.*, 18(21):6421 (1990)
X54223		AttB-related site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium glutamicum , and the attP site of lambdacorynephage," *FEMS. Microbiol. Lett.*, 66:299-302 (1990)
X54740	argS; lysA	Arginyl-tRNA synthetase; Diaminopimelate decarboxylase	Marcel, T. et al. "Nucleotide sequence and organization of the upstream region of the Corynebacterium glutamicum lysA gene," *Mol. Microbiol.*, 4(11):1819-1830 (1990)

Table 2 (continued)

X55994	trpL; trpE	Putative leader peptide; anthranilate synthase component I	Heery, D.M. et al. "Nucleotide sequence of the Corynebacterium glutamicum trpE gene," *Nucleic Acids Res.*, 18(23):7138 (1990)
X56037	thrC	Threonine synthase	Han, K.S. et al. "The molecular structure of the Corynebacterium glutamicum threonine synthase gene," *Mol. Microbiol.*, 4(10):1693-1702 (1990)
X56075	attB-related site	Attachment site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium glutamicum , and the attP site of lambdacorynephage," *FEMS. Microbiol, Lett.*, 66:299-302 (1990)
X57226	lysC-alpha; lysC-beta; asd	Aspartokinase-alpha subunit; Aspartokinase-beta subunit; aspartate beta semialdehyde dehydrogenase	Kalinowski, J. et al. "Genetic and biochemical analysis of the Aspartokinase from Corynebacterium glutamicum," *Mol. Microbiol.*, 5(5):1197-1204 (1991); Kalinowski, J. et al. "Aspartokinase genes lysC alpha and lysC beta overlap and are adjacent to the aspertate beta-semialdehyde dehydrogenase gene asd in Corynebacterium glutamicum," *Mol. Gen. Genet.*, 224(3):317-324 (1990)
X59403	gap;pgk; tpi	Glyceraldehyde-3-phosphate; phosphoglycerate kinase; triosephosphate isomerase	Eikmanns, B.J. "Identification, sequence analysis, and expression of a Corynebacterium glutamicum gene cluster encoding the three glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and triosephosphate isomeras," *J. Bacteriol.*, 174(19):6076-6086 (1992)
X59404	gdh	Glutamate dehydrogenase	Bormann, E.R. et al. "Molecular analysis of the Corynebacterium glutamicum gdh gene encoding glutamate dehydrogenase," *Mol. Microbiol.*, 6(3):317-326 (1992)
X60312	lysI	L-lysine permease	Seep-Feldhaus, A.H. et al. "Molecular analysis of the Corynebacterium glutamicum lysI gene involved in lysine uptake," *Mol. Microbiol.*, 5(12):2995-3005 (1991)
X66078	cop1	Ps1 protein	Joliff, G. et al. "Cloning and nucleotide sequence of the csp1 gene encoding PS1, one of the two major secreted proteins of Corynebacterium glutamicum: The deduced N-terminal region of PS1 is similar to the Mycobacterium antigen 85 complex," *Mol. Microbiol.*, 6(16):2349-2362 (1992)
X66112	glt	Citrate synthase	Eikmanns, B.J. et al. "Cloning sequence, expression and transcriptional analysis of the Corynebacterium glutamicum gltA gene encoding citrate synthase," *Microbiol.*, 140:1817-1828 (1994)
X67737	dapB	Dihydrodipicolinate reductase	
X69103	csp2	Surface layer protein PS2	Peyret, J.L. et al. "Characterization of the cspB gene encoding PS2, an ordered surface-layer protein in Corynebacterium glutamicum," *Mol. Microbiol.*, 9(1):97-109 (1993)
X69104		IS3 related insertion element	Bonamy, C. et al. "Identification of IS1206, a Corynebacterium glutamicum IS3-related insertion sequence and phylogenetic analysis," *Mol. Microbiol.*, 14(3):571-581 (1994)

Table 2 (continued)

X70959	leuA	Isopropylmalate synthase	Patek, M. et al. "Leucine synthesis in Corynebacterium glutamicum: enzyme activities, structure of leuA, and effect of leuA inactivation on lysine synthesis," *Appl. Environ. Microbiol.*, 60(1):133-140 (1994)
X71489	icd	Isocitrate dehydrogenase (NADP+)	Eikmanns, B.J. et al. "Cloning sequence analysis, expression, and inactivation of the Corynebacterium glutamicum icd gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme," *J. Bacteriol.*, 177(3):774-782 (1995)
X72855	GDHA	Glutamate dehydrogenase (NADP+)	
X75083, X70584	mtrA	5-methyltryptophan resistance	Heery, D.M. et al. "A sequence from a tryptophan-hyperproducing strain of Corynebacterium glutamicum encoding resistance to 5-methyltryptophan," *Biochem. Biophys. Res. Commun.*, 201(3):1255-1262 (1994)
X75085	recA		Fitzpatrick, R. et al. "Construction and characterization of recA mutant strains of Corynebacterium glutamicum and Brevibacterium lactofermentum," *Appl. Microbiol. Biotechnol.*, 42(4):575-580 (1994)
X75504	aceA; thiX	Partial Isocitrate lyase; ?	Reinscheid, D.J. et al. "Characterization of the isocitrate lyase gene from Corynebacterium glutamicum and biochemical analysis of the enzyme," *J. Bacteriol.*, 176(12):3474-3483 (1994)
X76875		ATPase beta-subunit	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes," *Antonie Van Leeuwenhoek*, 64:285-305 (1993)
X77034	tuf	Elongation factor Tu	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes," *Antonie Van Leeuwenhoek*, 64:285-305 (1993)
X77384	recA		Billman-Jacobe, H. "Nucleotide sequence of a recA gene from Corynebacterium glutamicum," *DNA Seq.*, 4(6):403-404 (1994)
X78491	aceB	Malate synthase	Reinscheid, D.J. et al. "Malate synthase from Corynebacterium glutamicum pta-ack operon encoding phosphotransacetylase: sequence analysis," *Microbiology*, 140:3099-3108 (1994)
X80629	16S rDNA	16S ribosomal RNA	Rainey, F.A. et al. "Phylogenetic analysis of the genera Rhodococcus and Norcardia and evidence for the evolutionary origin of the genus Norcardia from within the radiation of Rhodococcus species," *Microbiol.*, 141:523-528 (1995)
X81191	gluA; gluB; gluC; gluD	Glutamate uptake system	Kronemeyer, W. et al. "Structure of the gluABCD cluster encoding the glutamate uptake system of Corynebacterium glutamicum," *J. Bacteriol.*, 177(5):1152-1158 (1995)
X81379	dapE	Succinyldiaminopimelate desuccinylase	Wehrmann, A. et al. "Analysis of different DNA fragments of Corynebacterium glutamicum complementing dapE of Escherichia coli," *Microbiology*, 40:3349-56 (1994)

Table 2 (continued)

X82061	16S rDNA	16S ribosomal RNA	Ruimy, R. et al. "Phylogeny of the genus Corynebacterium deduced from analyses of small-subunit ribosomal DNA sequences," *Int. J. Syst. Bacteriol.*, 45(4):740-746 (1995)
X82928	asd; lysC	Aspartate-semialdehyde dehydrogenase; ?	Serebrijski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," *J. Bacteriol.*, 177(24):7255-7260 (1995)
X82929	proA	Gamma-glutamyl phosphate reductase	Serebrijski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," *J. Bacteriol.*, 177(24):7255-7260 (1995)
X84257	16S rDNA	16S ribosomal RNA	Pascual, C. et al. "Phylogenetic analysis of the genus Corynebacterium based on 16S rRNA gene sequences," *Int. J. Syst. Bacteriol.*, 45(4):724-728 (1995)
X85965	aroP; dapE	Aromatic amino acid permease; ?	Wehrmann et al. "Functional analysis of sequences adjacent to dapE of C. glutamicum proline reveals the presence of aroP, which encodes the aromatic amino acid transporter," *J. Bacteriol.*, 177(20):5991-5993 (1995)
X86157	argB; argC; argD; argF; argJ	Acetylglutamate kinase; N-acetyl-gamma-glutamyl-phosphate reductase; acetylornithine aminotransferase; ornithine carbamoyltransferase; glutamate N-acetyltransferase	Sakanyan, V. et al. "Genes and enzymes of the acetyl cycle of arginine biosynthesis in Corynebacterium glutamicum: enzyme evolution in the early steps of the arginine pathway," *Microbiology*, 142:99-108 (1996)
X89084	pta; ackA	Phosphate acetyltransferase; acetate kinase	Reinscheid, D.J. et al. "Cloning, sequence analysis, expression and inactivation of the Corynebacterium glutamicum pta-ack operon encoding phosphotransacetylase and acetate kinase," *Microbiology*, 145:503-513 (1999)
X89850	attB	Attachment site	Le Marrec, C. et al. "Genetic characterization of site-specific integration functions of phi AAU2 infecting "Arthrobacter aureus C70," *J. Bacteriol.*, 178(7):1996-2004 (1996)
X90356		Promoter fragment F1	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90357		Promoter fragment F2	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90358		Promoter fragment F10	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90359		Promoter fragment F13	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)

Table 2 (continued)

X90360		Promoter fragment F22	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90361		Promoter fragment F34	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90362		Promoter fragment F37	Patek, M. et al. "Promoters from C. glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90363		Promoter fragment F45	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90364		Promoter fragment F64	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90365		Promoter fragment F75	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90366		Promoter fragment PF101	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90367		Promoter fragment PF104	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90368		Promoter fragment PF109	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X93513	amt	Ammonium transport system	Siewe, R.M. et al. "Functional and genetic characterization of the (methyl) ammonium uptake carrier of Corynebacterium glutamicum," *J. Biol. Chem.*, 271(10):5398-5403 (1996)
X93514	betP	Glycine betaine transport system	Peter, H. et al. "Isolation, characterization, and expression of the Corynebacterium glutamicum betP gene, encoding the transport system for the compatible solute glycine betaine," *J. Bacteriol.*, 178(17):5229-5234 (1996)
X95649	orf4		Patek, M. et al. "Identification and transcriptional analysis of the dapB-ORF2-dapA-ORF4 operon of Corynebacterium glutamicum, encoding two enzymes involved in L-lysine synthesis," *Biotechnol. Lett.*, 19:1113-1117 (1997)
X96471	lysE; lysG	Lysine exporter protein; Lysine export regulator protein	Vrljic, M. et al. "A new type of transporter with a new type of cellular function: L-lysine export from Corynebacterium glutamicum," *Mol. Microbiol.*, 22(5):815-826 (1996)

Table 2 (continued)

X96580	panB; panC; xylB	3-methyl-2-oxobutanoate hydroxymethyltransferase; pantoate-beta-alanine ligase; xylulokinase	Sahm, H. et al. "D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction," *Appl. Environ. Microbiol.*, 65(5):1973-1979 (1999)
X96962		Insertion sequence IS1207 and transposase	
X99289		Elongation factor P	Ramos, A. et al. "Cloning, sequencing and expression of the gene encoding elongation factor P in the amino-acid producer Brevibacterium lactofermentum (Corynebacterium glutamicum ATCC 13869)," *Gene*, 198:217-222 (1997)
Y00140	thrB	Homoserine kinase	Mateos, L.M. et al. "Nucleotide sequence of the homoserine kinase (thrB) gene of the Brevibacterium lactofermentum," *Nucleic Acids Res.*, 15(9):3922 (1987)
Y00151	ddh	Meso-diaminopimelate D-dehydrogenase (EC 1.4.1.16)	Ishino, S. et al. "Nucleotide sequence of the meso-diaminopimelate D-dehydrogenase gene from Corynebacterium glutamicum," *Nucleic Acids Res.*, 15(9):3917 (1987)
Y00476	thrA	Homoserine dehydrogenase	Mateos, L.M. et al. "Nucleotide sequence of the homoserine dehydrogenase (thrA) gene of the Brevibacterium lactofermentum," *Nucleic Acids Res.*, 15(24):10598 (1987)
Y00546	hom; thrB	Homoserine dehydrogenase; homoserine kinase	Peoples, O.P. et al. "Nucleotide sequence and fine structural analysis of the Corynebacterium glutamicum hom-thrB operon," *Mol. Microbiol.*, 2(1):63-72 (1988)
Y08964	murC; ftsQ/divD; ftsZ	UPD-N-acetylmuramate-alanine ligase; division initiation protein or cell division protein; cell division protein	Honrubia, M.P. et al. "Identification, characterization, and chromosomal organization of the ftsZ gene from Brevibacterium lactofermentum," *Mol. Gen. Genet.*, 259(1):97-104 (1998)
Y09163	putP	High affinity proline transport system	Peter, H. et al. "Isolation of the putP gene of Corynebacterium glutamicumproline and characterization of a low-affinity uptake system for compatible solutes," *Arch. Microbiol.*, 168(2):143-151 (1997)
Y09548	pyc	Pyruvate carboxylase	Peters-Wendisch, P.G. et al. "Pyruvate carboxylase from Corynebacterium glutamicum: characterization, expression and inactivation of the pyc gene," *Microbiology*, 144:915-927 (1998)
Y09578	leuB	3-isopropylmalate dehydrogenase	Patek, M. et al. "Analysis of the leuB gene from Corynebacterium glutamicum," *Appl. Microbiol. Biotechnol.*, 50(1):42-47 (1998)
Y12472		Attachment site bacteriophage Phi-16	Moreau, S. et al. "Site-specific integration of corynephage Phi-16: The construction of an integration vector," *Microbiol.*, 145:539-548 (1999)
Y12537	proP	Proline/ectoine uptake system protein	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes: Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP," *J. Bacteriol.*, 180(22):6005-6012 (1998)

Table 2 (continued)

Y13221	glnA	Glutamine synthetase I	Jakoby, M. et al. "Isolation of Corynebacterium glutamicum glnA gene encoding glutamine synthetase I," *FEMS Microbiol. Lett.*, 154(1):81-88 (1997)
Y16642	lpd	Dihydrolipoamide dehydrogenase	
Y18059		Attachment site Corynephage 304L	Moreau, S. et al. "Analysis of the integration functions of φ304L: An integrase module among corynephages," *Virology*, 255(1):150-159 (1999)
Z21501	argS; lysA	Arginyl-tRNA synthetase; diaminopimelate decarboxylase (partial)	Oguiza, J.A. et al. "A gene encoding arginyl-tRNA synthetase is located in the upstream region of the lysA gene in Brevibacterium lactofermentum: Regulation of argS-lysA cluster expression by arginine," *J. Bacteriol.*,175(22):7356-7362 (1993)
Z21502	dapA; dapB	Dihydrodipicolinate synthase; dihydrodipicolinate reductase	Pisabarro, A. et al. "A cluster of three genes (dapA, orf2, and dapB) of Brevibacterium lactofermentum encodes dihydrodipicolinate reductase, and a third polypeptide of unknown function," *J. Bacteriol.*, 175(9):2743-2749 (1993)
Z29563	thrC	Threonine synthase	Malumbres, M. et al. "Analysis and expression of the thrC gene of the encoded threonine synthase," *Appl. Environ. Microbiol.*, 60(7)2209-2219 (1994)
Z46753	16S rDNA	Gene for 16S ribosomal RNA	
Z49822	sigA	SigA sigma factor	Oguiza, J.A. et al "Multiple sigma factor genes in Brevibacterium lactofermentum: Characterization of sigA and sigB," *J. Bacteriol.*, 178(2):550-553 (1996)
Z49823	galE; dtxR	Catalytic activity UDP-galactose 4-epimerase; diphtheria toxin regulatory protein	Oguiza, J.A. et al "The galE gene encoding the UDP-galactose 4-epimerase of Brevibacterium lactofermentum is coupled transcriptionally to the dmdR gene," *Gene*, 177:103-107 (1996)
Z49824	orf1; sigB	?; SigB sigma factor	Oguiza, J.A. et al "Multiple sigma factor genes in Brevibacterium lactofermentum: Characterization of sigA and sigB," *J. Bacteriol.*, 178(2):550-553 (1996)
Z66534		Transposase	Correia, A. et al. "Cloning and characterization of an IS-like element present in the genome of Brevibacterium lactofermentum ATCC 13869," *Gene*, 170(1):91-94 (1996)

[1] A sequence for this gene was published in the indicated reference. However, the sequence obtained by the inventors of the present application is significantly longer than the published version. It is believed that the published version relied on an incorrect start codon, and thus represents only a fragment of the actual coding region.

Exemplification

Example 1: Preparation of total genomic DNA of *Corynebacterium glutamicum* ATCC 13032

A culture of *Corynebacterium glutamicum* (ATCC 13032) was grown overnight at 30°C with vigorous shaking in BHI medium (Difco). The cells were harvested by centrifugation, the supernatant was discarded and the cells were resuspended in 5 ml buffer-I (5% of the original volume of the culture — all indicated volumes have been calculated for 100 ml of culture volume). Composition of buffer-I: 140.34 g/l sucrose, 2.46 g/l $MgSO_4 \times 7H_2O$, 10 ml/l KH_2PO_4 solution (100 g/l, adjusted to pH 6.7 with KOH), 50 ml/l M12 concentrate (10 g/l $(NH_4)_2SO_4$, 1 g/l NaCl, 2 g/l $MgSO_4 \times 7H_2O$, 0.2 g/l $CaCl_2$, 0.5 g/l yeast extract (Difco), 10 ml/l trace-elements-mix (200 mg/l $FeSO_4 \times H_2O$, 10 mg/l $ZnSO_4 \times 7\ H_2O$, 3 mg/l $MnCl_2 \times 4\ H_2O$, 30 mg/l H_3BO, 20 mg/l $CoCl_2 \times 6\ H_2O$, 1 mg/l $NiCl_2 \times 6\ H_2O$, 3 mg/l $Na_2MoO_4 \times 2\ H_2O$, 500 mg/l complexing agent (EDTA or critic acid), 100 ml/l vitamins-mix (0.2 mg/l biotin, 0.2 mg/l folic acid, 20 mg/l p-amino benzoic acid, 20 mg/l riboflavin, 40 mg/l ca-panthothenate, 140 mg/l nicotinic acid, 40 mg/l pyridoxole hydrochloride, 200 mg/l myo-inositol). Lysozyme was added to the suspension to a final concentration of 2.5 mg/ml. After an approximately 4 h incubation at 37°C, the cell wall was degraded and the resulting protoplasts are harvested by centrifugation. The pellet was washed once with 5 ml buffer-I and once with 5 ml TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The pellet was resuspended in 4 ml TE-buffer and 0.5 ml SDS solution (10%) and 0.5 ml NaCl solution (5 M) are added. After adding of proteinase K to a final concentration of 200 µg/ml, the suspension is incubated for ca.18 h at 37°C. The DNA was purified by extraction with phenol, phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol using standard procedures. Then, the DNA was precipitated by adding 1/50 volume of 3 M sodium acetate and 2 volumes of ethanol, followed by a 30 min incubation at -20°C and a 30 min centrifugation at 12,000 rpm in a high speed centrifuge using a SS34 rotor (Sorvall). The DNA was dissolved in 1 ml TE-buffer containing 20 µg/ml RNaseA and dialysed at 4°C against 1000 ml TE-buffer for at least 3 hours. During this time, the buffer was exchanged 3 times. To aliquots of 0.4 ml of the dialysed DNA solution, 0.4 ml of 2 M LiCl and 0.8 ml of ethanol are added. After a 30

min incubation at -20°C, the DNA was collected by centrifugation (13,000 rpm, Biofuge Fresco, Heraeus, Hanau, Germany). The DNA pellet was dissolved in TE-buffer. DNA prepared by this procedure could be used for all purposes, including southern blotting or construction of genomic libraries.

Example 2: Construction of genomic libraries in *Escherichia coli* of *Corynebacterium glutamicum* ATCC13032.

Using DNA prepared as described in Example 1, cosmid and plasmid libraries were constructed according to known and well established methods (*see e.g.*, Sambrook, J. *et al.* (1989) "Molecular Cloning : A Laboratory Manual", Cold Spring Harbor Laboratory Press, or Ausubel, F.M. *et al.* (1994) "Current Protocols in Molecular Biology", John Wiley & Sons.)

Any plasmid or cosmid could be used. Of particular use were the plasmids pBR322 (Sutcliffe, J.G. (1979) *Proc. Natl. Acad. Sci. USA*, 75:3737-3741); pACYC177 (Change & Cohen (1978) *J. Bacteriol* 134:1141-1156), plasmids of the pBS series (pBSSK+, pBSSK- and others; Stratagene, LaJolla, USA), or cosmids as SuperCos1 (Stratagene, LaJolla, USA) or Lorist6 (Gibson, T.J., Rosenthal A. and Waterson, R.H. (1987) *Gene* 53:283-286. Gene libraries specifically for use in *C. glutamicum* may be constructed using plasmid pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol. Biotechnol.* 4: 256-263).

Example 3: DNA Sequencing and Computational Functional Analysis

Genomic libraries as described in Example 2 were used for DNA sequencing according to standard methods, in particular by the chain termination method using ABI377 sequencing machines (see *e.g.*, Fleischman, R.D. *et al.* (1995) "Whole-genome Random Sequencing and Assembly of Haemophilus Influenzae Rd., *Science*, 269:496-512). Sequencing primers with the following nucleotide sequences were used: 5'-GGAAACAGTATGACCATG-3' or 5'-GTAAAACGACGGCCAGT-3'.

Example 4: *In vivo* Mutagenesis

In vivo mutagenesis of *Corynebacterium glutamicum* can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (*e.g. Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae*) which are impaired in their capabilities to maintain

the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (*e.g.*, mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D. (1996) DNA repair mechanisms, in: *Escherichia coli* and *Salmonella*, p. 2277-2294, ASM: Washington.) Such strains are well known to those of ordinary skill in the art. The use of such strains is illustrated, for example, in Greener, A. and Callahan, M. (1994) *Strategies* 7: 32-34.

Example 5: DNA Transfer Between *Escherichia coli* and *Corynebacterium glutamicum*

Several *Corynebacterium* and *Brevibacterium* species contain endogenous plasmids (as *e.g.*, pHM1519 or pBL1) which replicate autonomously (for review see, *e.g.*, Martin, J.F. *et al.* (1987) *Biotechnology*, 5:137-146). Shuttle vectors for *Escherichia coli* and *Corynebacterium glutamicum* can be readily constructed by using standard vectors for *E. coli* (Sambrook, J. *et al.* (1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press or Ausubel, F.M. *et al.* (1994) "Current Protocols in Molecular Biology", John Wiley & Sons) to which a origin or replication for and a suitable marker from *Corynebacterium glutamicum* is added. Such origins of replication are preferably taken from endogenous plasmids isolated from *Corynebacterium* and *Brevibacterium* species. Of particular use as transformation markers for these species are genes for kanamycin resistance (such as those derived from the Tn5 or Tn903 transposons) or chloramphenicol (Winnacker, E.L. (1987) "From Genes to Clones — Introduction to Gene Technology, VCH, Weinheim). There are numerous examples in the literature of the construction of a wide variety of shuttle vectors which replicate in both *E. coli and C. glutamicum*, and which can be used for several purposes, including gene over-expression (for reference, see *e.g.*, Yoshihama, M. *et al.* (1985) *J. Bacteriol.* 162:591-597, Martin J.F. *et al.* (1987) *Biotechnology*, 5:137-146 and Eikmanns, B.J. *et al.* (1991) *Gene*, 102:93-98).

Using standard methods, it is possible to clone a gene of interest into one of the shuttle vectors described above and to introduce such a hybrid vectors into strains of *Corynebacterium glutamicum*. Transformation of *C. glutamicum* can be achieved by protoplast transformation (Kastsumata, R. *et al.* (1984) *J. Bacteriol.* 159306-311), electroporation (Liebl, E. *et al.* (1989) *FEMS Microbiol. Letters*, 53:399-303) and in cases where special vectors are used, also by conjugation (as described *e.g.* in Schäfer, A *et al.*

(1990) *J. Bacteriol.* 172:1663-1666). It is also possible to transfer the shuttle vectors for *C. glutamicum* to *E. coli* by preparing plasmid DNA from *C. glutamicum* (using standard methods well-known in the art) and transforming it into *E. coli*. This transformation step can be performed using standard methods, but it is advantageous to use an Mcr-deficient *E. coli* strain, such as NM522 (Gough & Murray (1983) *J. Mol. Biol.* 166:1-19).

Genes may be overexpressed in *C. glutamicum* strains using plasmids which comprise pCG1 (U.S. Patent No. 4,617,267) or fragments thereof, and optionally the gene for kanamycin resistance from TN903 (Grindley, N.D. and Joyce, C.M. (1980) *Proc. Natl. Acad. Sci. USA* 77(12): 7176-7180). In addition, genes may be overexpressed in *C. glutamicum* strains using plasmid pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol. Biotechnol.* 4: 256-263).

Aside from the use of replicative plasmids, gene overexpression can also be achieved by integration into the genome. Genomic integration in *C. glutamicum* or other Corynebacterium or Brevibacterium species may be accomplished by well-known methods, such as homologous recombination with genomic region(s), restriction endonuclease mediated integration (REMI) (see, *e.g.*, DE Patent 19823834), or through the use of transposons. It is also possible to modulate the activity of a gene of interest by modifying the regulatory regions (*e.g.*, a promoter, a repressor, and/or an enhancer) by sequence modification, insertion, or deletion using site-directed methods (such as homologous recombination) or methods based on random events (such as transposon mutagenesis or REMI). Nucleic acid sequences which function as transcriptional terminators may also be inserted 3' to the coding region of one or more genes of the invention; such terminators are well-known in the art and are described, for example, in Winnacker, E.L. (1987) From Genes to Clones – Introduction to Gene Technology. VCH: Weinheim.

Example 6: Assessment of the Expression of the Mutant Protein

Observations of the activity of a mutated protein in a transformed host cell rely on the fact that the mutant protein is expressed in a similar fashion and in a similar quantity to that of the wild-type protein. A useful method to ascertain the level of transcription of the mutant gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot (for reference see, for example, Ausubel *et al.*

(1988) Current Protocols in Molecular Biology, Wiley: New York), in which a primer designed to bind to the gene of interest is labeled with a detectable tag (usually radioactive or chemiluminescent), such that when the total RNA of a culture of the organism is extracted, run on gel, transferred to a stable matrix and incubated with this probe, the binding and quantity of binding of the probe indicates the presence and also the quantity of mRNA for this gene. This information is evidence of the degree of transcription of the mutant gene. Total cellular RNA can be prepared from *Corynebacterium glutamicum* by several methods, all well-known in the art, such as that described in Bormann, E.R. *et al.* (1992) *Mol. Microbiol.* 6: 317-326.

To assess the presence or relative quantity of protein translated from this mRNA, standard techniques, such as a Western blot, may be employed (see, for example, Ausubel *et al.* (1988) Current Protocols in Molecular Biology, Wiley: New York). In this process, total cellular proteins are extracted, separated by gel electrophoresis, transferred to a matrix such as nitrocellulose, and incubated with a probe, such as an antibody, which specifically binds to the desired protein. This probe is generally tagged with a chemiluminescent or colorimetric label which may be readily detected. The presence and quantity of label observed indicates the presence and quantity of the desired mutant protein present in the cell.

Example 7: Growth of Genetically Modified *Corynebacterium glutamicum* — Media and Culture Conditions

Genetically modified *Corynebacteria* are cultured in synthetic or natural growth media. A number of different growth media for Corynebacteria are both well-known and readily available (Lieb *et al.* (1989) *Appl. Microbiol. Biotechnol.*, 32:205-210; von der Osten *et al.* (1998) *Biotechnology Letters*, 11:11-16; Patent DE 4,120,867; Liebl (1992) "The Genus *Corynebacterium*, in: The Procaryotes, Volume II, Balows, A. *et al.*, eds. Springer-Verlag). These media consist of one or more carbon sources, nitrogen sources, inorganic salts, vitamins and trace elements. Preferred carbon sources are sugars, such as mono-, di-, or polysaccharides. For example, glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose serve as very good carbon sources. It is also possible to supply sugar to the media via complex compounds such as molasses or other by-products from sugar refinement. It can also be

advantageous to supply mixtures of different carbon sources. Other possible carbon sources are alcohols and organic acids, such as methanol, ethanol, acetic acid or lactic acid. Nitrogen sources are usually organic or inorganic nitrogen compounds, or materials which contain these compounds. Exemplary nitrogen sources include ammonia gas or ammonia salts, such as NH_4Cl or $(NH_4)_2SO_4$, NH_4OH, nitrates, urea, amino acids or complex nitrogen sources like corn steep liquor, soy bean flour, soy bean protein, yeast extract, meat extract and others.

Inorganic salt compounds which may be included in the media include the chloride-, phosphorous- or sulfate- salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron. Chelating compounds can be added to the medium to keep the metal ions in solution. Particularly useful chelating compounds include dihydroxyphenols, like catechol or protocatechuate, or organic acids, such as citric acid. It is typical for the media to also contain other growth factors, such as vitamins or growth promoters, examples of which include biotin, riboflavin, thiamin, folic acid, nicotinic acid, pantothenate and pyridoxin. Growth factors and salts frequently originate from complex media components such as yeast extract, molasses, corn steep liquor and others. The exact composition of the media compounds depends strongly on the immediate experiment and is individually decided for each specific case. Information about media optimization is available in the textbook "Applied Microbiol. Physiology, A Practical Approach (*eds.* P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). It is also possible to select growth media from commercial suppliers, like standard 1 (Merck) or BHI (grain heart infusion, DIFCO) or others.

All medium components are sterilized, either by heat (20 minutes at 1.5 bar and 121°C) or by sterile filtration. The components can either be sterilized together or, if necessary, separately. All media components can be present at the beginning of growth, or they can optionally be added continuously or batchwise.

Culture conditions are defined separately for each experiment. The temperature should be in a range between 15°C and 45°C. The temperature can be kept constant or can be altered during the experiment. The pH of the medium should be in the range of 5 to 8.5, preferably around 7.0, and can be maintained by the addition of buffers to the media. An exemplary buffer for this purpose is a potassium phosphate buffer. Synthetic buffers such as MOPS, HEPES, ACES and others can alternatively or simultaneously be used. It

is also possible to maintain a constant culture pH through the addition of NaOH or NH_4OH during growth. If complex medium components such as yeast extract are utilized, the necessity for additional buffers may be reduced, due to the fact that many complex compounds have high buffer capacities. If a fermentor is utilized for culturing the micro-organisms, the pH can also be controlled using gaseous ammonia.

The incubation time is usually in a range from several hours to several days. This time is selected in order to permit the maximal amount of product to accumulate in the broth. The disclosed growth experiments can be carried out in a variety of vessels, such as microtiter plates, glass tubes, glass flasks or glass or metal fermentors of different sizes. For screening a large number of clones, the microorganisms should be cultured in microtiter plates, glass tubes or shake flasks, either with or without baffles. Preferably 100 ml shake flasks are used, filled with 10% (by volume) of the required growth medium. The flasks should be shaken on a rotary shaker (amplitude 25 mm) using a speed-range of 100 – 300 rpm. Evaporation losses can be diminished by the maintenance of a humid atmosphere; alternatively, a mathematical correction for evaporation losses should be performed.

If genetically modified clones are tested, an unmodified control clone or a control clone containing the basic plasmid without any insert should also be tested. The medium is inoculated to an OD_{600} of 0.5 – 1.5 using cells grown on agar plates, such as CM plates (10 g/l glucose, 2,5 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l agar, pH 6.8 with 2M NaOH) that had been incubated at 30°C. Inoculation of the media is accomplished by either introduction of a saline suspension of *C. glutamicum* cells from CM plates or addition of a liquid preculture of this bacterium.

Example 8 – *In vitro* Analysis of the Function of Mutant Proteins

The determination of activities and kinetic parameters of enzymes is well established in the art. Experiments to determine the activity of any given altered enzyme must be tailored to the specific activity of the wild-type enzyme, which is well within the ability of one of ordinary skill in the art. Overviews about enzymes in general, as well as specific details concerning structure, kinetics, principles, methods, applications and examples for the determination of many enzyme activities may be

found, for example, in the following references: Dixon, M., and Webb, E.C., (1979) Enzymes. Longmans: London; Fersht, (1985) Enzyme Structure and Mechanism. Freeman: New York; Walsh, (1979) Enzymatic Reaction Mechanisms. Freeman: San Francisco; Price, N.C., Stevens, L. (1982) Fundamentals of Enzymology. Oxford Univ. Press: Oxford; Boyer, P.D., ed. (1983) The Enzymes, 3rd ed. Academic Press: New York; Bisswanger, H., (1994) Enzymkinetik, 2nd ed. VCH: Weinheim (ISBN 3527300325); Bergmeyer, H.U., Bergmeyer, J., Graßl, M., eds. (1983-1986) Methods of Enzymatic Analysis, 3rd ed., vol. I-XII, Verlag Chemie: Weinheim; and Ullmann's Encyclopedia of Industrial Chemistry (1987) vol. A9, "Enzymes". VCH: Weinheim, p. 352-363.

The activity of proteins which bind to DNA can be measured by several well-established methods, such as DNA band-shift assays (also called gel retardation assays). The effect of such proteins on the expression of other molecules can be measured using reporter gene assays (such as that described in Kolmar, H. *et al.* (1995) *EMBO J.* 14: 3895-3904 and references cited therein). Reporter gene test systems are well known and established for applications in both pro- and eukaryotic cells, using enzymes such as beta-galactosidase, green fluorescent protein, and several others.

The determination of activity of membrane-transport proteins can be performed according to techniques such as those described in Gennis, R.B. (1989) "Pores, Channels and Transporters", in Biomembranes, Molecular Structure and Function, Springer: Heidelberg, p. 85-137; 199-234; and 270-322.

Example 9: Analysis of Impact of Mutant Protein on the Production of the Desired Product

The effect of the genetic modification in *C. glutamicum* on production of a desired compound (such as an amino acid) can be assessed by growing the modified microorganism under suitable conditions (such as those described above) and analyzing the medium and/or the cellular component for increased production of the desired product (*i.e.*, an amino acid). Such analysis techniques are well known to one of ordinary skill in the art, and include spectroscopy, thin layer chromatography, staining methods of various kinds, enzymatic and microbiological methods, and analytical chromatography such as high performance liquid chromatography (see, for example,

Ullman, Encyclopedia of Industrial Chemistry, vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A. *et al.*, (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm *et al.* (1993) Biotechnology, vol. 3, Chapter III: "Product recovery and purification", page 469-714, VCH: Weinheim; Belter, P.A. *et al.* (1988) Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S. (1992) Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D. (1988) Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.)

In addition to the measurement of the final product of fermentation, it is also possible to analyze other components of the metabolic pathways utilized for the production of the desired compound, such as intermediates and side-products, to determine the overall efficiency of production of the compound. Analysis methods include measurements of nutrient levels in the medium (*e.g.*, sugars, hydrocarbons, nitrogen sources, phosphate, and other ions), measurements of biomass composition and growth, analysis of the production of common metabolites of biosynthetic pathways, and measurement of gasses produced during fermentation. Standard methods for these measurements are outlined in Applied Microbial Physiology, A Practical Approach, P.M. Rhodes and P.F. Stanbury, eds., IRL Press, p. 103-129; 131-163; and 165-192 (ISBN: 0199635773) and references cited therein.

Example 10: Purification of the Desired Product from *C. glutamicum* Culture

Recovery of the desired product from the *C. glutamicum* cells or supernatant of the above-described culture can be performed by various methods well known in the art. If the desired product is not secreted from the cells, the cells can be harvested from the culture by low-speed centrifugation, the cells can be lysed by standard techniques, such as mechanical force or sonication. The cellular debris is removed by centrifugation, and the supernatant fraction containing the soluble proteins is retained for further purification of the desired compound. If the product is secreted from the *C. glutamicum*

cells, then the cells are removed from the culture by low-speed centrifugation, and the supernate fraction is retained for further purification.

The supernatant fraction from either purification method is subjected to chromatography with a suitable resin, in which the desired molecule is either retained on a chromatography resin while many of the impurities in the sample are not, or where the impurities are retained by the resin while the sample is not. Such chromatography steps may be repeated as necessary, using the same or different chromatography resins. One of ordinary skill in the art would be well-versed in the selection of appropriate chromatography resins and in their most efficacious application for a particular molecule to be purified. The purified product may be concentrated by filtration or ultrafiltration, and stored at a temperature at which the stability of the product is maximized.

There are a wide array of purification methods known to the art and the preceding method of purification is not meant to be limiting. Such purification techniques are described, for example, in Bailey, J.E. & Ollis, D.F. Biochemical Engineering Fundamentals, McGraw-Hill: New York (1986).

The identity and purity of the isolated compounds may be assessed by techniques standard in the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin layer chromatography, NIRS, enzymatic assay, or microbiologically. Such analysis methods are reviewed in: Patek *et al.* (1994) *Appl. Environ. Microbiol.* 60: 133-140; Malakhova *et al.* (1996) *Biotekhnologiya* 11: 27-32; and Schmidt *et al.* (1998) *Bioprocess Engineer.* 19: 67-70. Ulmann's Encyclopedia of Industrial Chemistry, (1996) vol. A27, VCH: Weinheim, p. 89-90, p. 521-540, p. 540-547, p. 559-566, 575-581 and p. 581-587; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A. *et al.* (1987) Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17.

Example 11: Analysis of the Gene Sequences of the Invention

The comparison of sequences and determination of percent homology between two sequences are art-known techniques, and can be accomplished using a mathematical algorithm, such as the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci.* USA 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA

90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to SMP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to SMP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, one of ordinary skill in the art will know how to optimize the parameters of the program (*e.g.*, XBLAST and NBLAST) for the specific sequence being analyzed.

Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Meyers and Miller ((1988) *Comput. Appl. Biosci.* 4: 11-17). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art, and include ADVANCE and ADAM. described in Torelli and Robotti (1994) *Comput. Appl. Biosci.* 10:3-5; and FASTA, described in Pearson and Lipman (1988) *P.N.A.S.* 85:2444-8.

The percent homology between two amino acid sequences can also be accomplished using the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. The percent homology between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package, using standard parameters, such as a gap weight of 50 and a length weight of 3.

A comparative analysis of the gene sequences of the invention with those present in Genbank has been performed using techniques known in the art (see, *e.g.*, Bexevanis and Ouellette, eds. (1998) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins. John Wiley and Sons: New York). The gene sequences of the invention

were compared to genes present in Genbank in a three-step process. In a first step, a BLASTN analysis (*e.g.*, a local alignment analysis) was performed for each of the sequences of the invention against the nucleotide sequences present in Genbank, and the top 500 hits were retained for further analysis. A subsequent FASTA search (*e.g.*, a combined local and global alignment analysis, in which limited regions of the sequences are aligned) was performed on these 500 hits. Each gene sequence of the invention was subsequently globally aligned to each of the top three FASTA hits, using the GAP program in the GCG software package (using standard parameters). In order to obtain correct results, the length of the sequences extracted from Genbank were adjusted to the length of the query sequences by methods well-known in the art. The results of this analysis are set forth in Table 4. The resulting data is identical to that which would have been obtained had a GAP (global) analysis alone been performed on each of the genes of the invention in comparison with each of the references in Genbank, but required significantly reduced computational time as compared to such a database-wide GAP (global) analysis. Sequences of the invention for which no alignments above the cutoff values were obtained are indicated on Table 4 by the absence of alignment information. It will further be understood by one of ordinary skill in the art that the GAP alignment homology percentages set forth in Table 4 under the heading "% homology (GAP)" are listed in the European numerical format, wherein a ',' represents a decimal point. For example, a value of "40,345" in this column represents "40.345%".

Example 12: Construction and Operation of DNA Microarrays

The sequences of the invention may additionally be used in the construction and application of DNA microarrays (the design, methodology, and uses of DNA arrays are well known in the art, and are described, for example, in Schena, M. *et al.* (1995) *Science* 270: 467-470; Wodicka, L. *et al.* (1997) *Nature Biotechnology* 15: 1359-1367; DeSaizieu, A. *et al.* (1998) *Nature Biotechnology* 16: 45-48; and DeRisi, J.L. *et al.* (1997) *Science* 278: 680-686).

DNA microarrays are solid or flexible supports consisting of nitrocellulose, nylon, glass, silicone, or other materials. Nucleic acid molecules may be attached to the surface in an ordered manner. After appropriate labeling, other nucleic acids or nucleic acid mixtures can be hybridized to the immobilized nucleic acid molecules, and the label

may be used to monitor and measure the individual signal intensities of the hybridized molecules at defined regions. This methodology allows the simultaneous quantification of the relative or absolute amount of all or selected nucleic acids in the applied nucleic acid sample or mixture. DNA microarrays, therefore, permit an analysis of the expression of multiple (as many as 6800 or more) nucleic acids in parallel (see, *e.g.*, Schena, M. (1996) *BioEssays* 18(5): 427-431).

The sequences of the invention may be used to design oligonucleotide primers which are able to amplify defined regions of one or more *C. glutamicum* genes by a nucleic acid amplification reaction such as the polymerase chain reaction. The choice and design of the 5' or 3' oligonucleotide primers or of appropriate linkers allows the covalent attachment of the resulting PCR products to the surface of a support medium described above (and also described, for example, Schena, M. *et al.* (1995) *Science* 270: 467-470).

Nucleic acid microarrays may also be constructed by *in situ* oligonucleotide synthesis as described by Wodicka, L. *et al.* (1997) *Nature Biotechnology* 15: 1359-1367. By photolithographic methods, precisely defined regions of the matrix are exposed to light. Protective groups which are photolabile are thereby activated and undergo nucleotide addition, whereas regions that are masked from light do not undergo any modification. Subsequent cycles of protection and light activation permit the synthesis of different oligonucleotides at defined positions. Small, defined regions of the genes of the invention may be synthesized on microarrays by solid phase oligonucleotide synthesis.

The nucleic acid molecules of the invention present in a sample or mixture of nucleotides may be hybridized to the microarrays. These nucleic acid molecules can be labeled according to standard methods. In brief, nucleic acid molecules (*e.g.*, mRNA molecules or DNA molecules) are labeled by the incorporation of isotopically or fluorescently labeled nucleotides, *e.g.*, during reverse transcription or DNA synthesis. Hybridization of labeled nucleic acids to microarrays is described (*e.g.*, in Schena, M. *et al.* (1995) *supra*; Wodicka, L. *et al.* (1997), *supra*; and DeSaizieu A. *et al.* (1998), *supra*). The detection and quantification of the hybridized molecule are tailored to the specific incorporated label. Radioactive labels can be detected, for example, as

described in Schena, M. *et al.* (1995) *supra*) and fluorescent labels may be detected, for example, by the method of Shalon *et al.* (1996) *Genome Research* 6: 639-645).

The application of the sequences of the invention to DNA microarray technology, as described above, permits comparative analyses of different strains of *C. glutamicum* or other Corynebacteria. For example, studies of inter-strain variations based on individual transcript profiles and the identification of genes that are important for specific and/or desired strain properties such as pathogenicity, productivity and stress tolerance are facilitated by nucleic acid array methodologies. Also, comparisons of the profile of expression of genes of the invention during the course of a fermentation reaction are possible using nucleic acid array technology.

Example 13: Analysis of the Dynamics of Cellular Protein Populations (Proteomics)

The genes, compositions, and methods of the invention may be applied to study the interactions and dynamics of populations of proteins, termed 'proteomics'. Protein populations of interest include, but are not limited to, the total protein population of *C. glutamicum* (*e.g.*, in comparison with the protein populations of other organisms), those proteins which are active under specific environmental or metabolic conditions (*e.g.*, during fermentation, at high or low temperature, or at high or low pH), or those proteins which are active during specific phases of growth and development.

Protein populations can be analyzed by various well-known techniques, such as gel electrophoresis. Cellular proteins may be obtained, for example, by lysis or extraction, and may be separated from one another using a variety of electrophoretic techniques. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separates proteins largely on the basis of their molecular weight. Isoelectric focusing polyacrylamide gel electrophoresis (IEF-PAGE) separates proteins by their isoelectric point (which reflects not only the amino acid sequence but also posttranslational modifications of the protein). Another, more preferred method of protein analysis is the consecutive combination of both IEF-PAGE and SDS-PAGE, known as 2-D-gel electrophoresis (described, for example, in Hermann *et al.* (1998) *Electrophoresis* 19: 3217-3221; Fountoulakis *et al.* (1998) *Electrophoresis* 19: 1193-1202; Langen *et al.* (1997) *Electrophoresis* 18: 1184-1192; Antelmann *et al.* (1997) *Electrophoresis* 18:

1451-1463). Other separation techniques may also be utilized for protein separation, such as capillary gel electrophoresis; such techniques are well known in the art.

Proteins separated by these methodologies can be visualized by standard techniques, such as by staining or labeling. Suitable stains are known in the art, and include Coomassie Brilliant Blue, silver stain, or fluorescent dyes such as Sypro Ruby (Molecular Probes). The inclusion of radioactively labeled amino acids or other protein precursors (*e.g.*, ^{35}S-methionine, ^{35}S-cysteine, ^{14}C-labelled amino acids, ^{15}N-amino acids, $^{15}NO_3$ or $^{15}NH_4^+$ or ^{13}C-labelled amino acids) in the medium of *C. glutamicum* permits the labeling of proteins from these cells prior to their separation. Similarly, fluorescent labels may be employed. These labeled proteins can be extracted, isolated and separated according to the previously described techniques.

Proteins visualized by these techniques can be further analyzed by measuring the amount of dye or label used. The amount of a given protein can be determined quantitatively using, for example, optical methods and can be compared to the amount of other proteins in the same gel or in other gels. Comparisons of proteins on gels can be made, for example, by optical comparison, by spectroscopy, by image scanning and analysis of gels, or through the use of photographic films and screens. Such techniques are well-known in the art.

To determine the identity of any given protein, direct sequencing or other standard techniques may be employed. For example, N- and/or C-terminal amino acid sequencing (such as Edman degradation) may be used, as may mass spectrometry (in particular MALDI or ESI techniques (see, *e.g.*, Langen *et al.* (1997) *Electrophoresis* 18: 1184-1192)). The protein sequences provided herein can be used for the identification of *C. glutamicum* proteins by these techniques.

The information obtained by these methods can be used to compare patterns of protein presence, activity, or modification between different samples from various biological conditions (*e.g.*, different organisms, time points of fermentation, media conditions, or different biotopes, among others). Data obtained from such experiments alone, or in combination with other techniques, can be used for various applications, such as to compare the behavior of various organisms in a given (*e.g.*, metabolic) situation, to increase the productivity of strains which produce fine chemicals or to increase the efficiency of the production of fine chemicals.

Equivalents

Those of ordinary skill in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule from *Corynebacterium glutamicum* encoding an SMP protein, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

2. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes an SMP protein involved in the production of a fine chemical.

3. An isolated *Corynebacterium glutamicum* nucleic acid molecule selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

4. An isolated nucleic acid molecule which encodes a polypeptide sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing,, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

5. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide selected from the group of amino acid sequences consisting of those sequences set forth in as even-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

6. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

7. An isolated nucleic acid molecule comprising a fragment of at least 15 nucleotides of a nucleic acid comprising a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

8. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1-7 under stringent conditions.

9. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1-8 or a portion thereof and a nucleotide sequence encoding a heterologous polypeptide.

10. A vector comprising the nucleic acid molecule of any one of claims 1-9.

11. The vector of claim 10, which is an expression vector.

12. A host cell transfected with the expression vector of claim 11.

13. The host cell of claim 12, wherein said cell is a microorganism.

14. The host cell of claim 13, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.

15. The host cell of claim 12, wherein the expression of said nucleic acid molecule results in the modulation in production of a fine chemical from said cell.

16. The host cell of claim 15, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.

17. A method of producing a polypeptide comprising culturing the host cell of claim 12 in an appropriate culture medium to, thereby, produce the polypeptide.

18. An isolated SMP polypeptide from *Corynebacterium glutamicum*, or a portion thereof.

19. The polypeptide of claim 18, wherein said polypeptide is involved in the production of a fine chemical.

20. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.

21. An isolated polypeptide comprising a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.

22. The isolated polypeptide of any of claims 18-21, further comprising heterologous amino acid sequences.

23. An isolated polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleic acid selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated nucleic acid molecules set forth in Table 1.

24. An isolated polypeptide comprising an amino acid sequence which is at least 50% homologous to an amino acid sequence selected from the group consisting of those

sequences as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.

25. A method for producing a fine chemical, comprising culturing a cell containing a vector of claim 12 such that the fine chemical is produced.

26. The method of claim 25, wherein said method further comprises the step of recovering the fine chemical from said culture.

27. The method of claim 25, wherein said method further comprises the step of transfecting said cell with the vector of claim 11 to result in a cell containing said vector.

28. The method of claim 25, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.

29. The method of claim 25, wherein said cell is selected from the group consisting of: *Corynebacterium glutamicum, Corynebacterium herculis, Corynebacterium, lilium, Corynebacterium acetoacidophilum, Corynebacterium acetoglutamicum, Corynebacterium acetophilum, Corynebacterium ammoniagenes, Corynebacterium fujiokense, Corynebacterium nitrilophilus, Brevibacterium ammoniagenes, Brevibacterium butanicum, Brevibacterium divaricatum, Brevibacterium flavum, Brevibacterium healii, Brevibacterium ketoglutamicum, Brevibacterium ketosoreductum, Brevibacterium lactofermentum, Brevibacterium linens, Brevibacterium paraffinolyticum*, and those strains set forth in Table 3.

30. The method of claim 25, wherein expression of the nucleic acid molecule from said vector results in modulation of production of said fine chemical.

31. The method of claim 25, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine

and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.

32. The method of claim 25, wherein said fine chemical is an amino acid.

33. The method of claim 32, wherein said amino acid is drawn from the group consisting of: lysine, glutamate, glutamine, alanine, aspartate, glycine, serine, threonine, methionine, cysteine, valine, leucine, isoleucine, arginine, proline, histidine, tyrosine, phenylalanine, and tryptophan.

34. A method for producing a fine chemical, comprising culturing a cell whose genomic DNA has been altered by the inclusion of a nucleic acid molecule of any one of claims 1-9.

35. A method for diagnosing the presence or activity of *Corynebacterium diphtheriae* in a subject, comprising detecting the presence of one or more of SEQ ID NOs 1 through 782 of the Sequence Listing in the subject, provided that the sequences are not or are not encoded by any of the F-designated sequences set forth in Table 1, thereby diagnosing the presence or activity of *Corynebacterium diphtheriae* in the subject.

36. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the nucleic acid molecule is disrupted.

35. 37. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs in the Sequence Listing, wherein the nucleic acid molecule comprises one or more nucleic acid modifications from the sequence set forth as odd-numbered SEQ ID NOs of the Sequence Listing s.

38. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the regulatory region of the nucleic acid molecule is modified relative to the wild-type regulatory region of the molecule.

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Met Arg Gln Ala Leu Asp Ala Gly Pro Ser Phe Phe Gln Lys Ala His
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Leu Asp Leu Ala Ser Leu Ser Ile Ala Glu Phe Tyr Glu Asp Gly Pro
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Phe Thr Gly Trp Val Asp Val Asn Leu Thr Glu Gln Gly Glu Ala Glu
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gcc aaa ggc gtc ctc cca ggc gtt gta tac acc tcc ttg ctg cgt cgc   259
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atc cca gtg atc cgc gac tgg cgc ctc aac gag cgt cac tac ggc gca   355
Ile Pro Val Ile Arg Asp Trp Arg Leu Asn Glu Arg His Tyr Gly Ala
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ctg cag ggc ctt gac aag gct gca acc aag gaa aaa tac ggc gac gac   403
Leu Gln Gly Leu Asp Lys Ala Ala Thr Lys Glu Lys Tyr Gly Asp Asp
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gcg gat gac gca gag tac tcc cag gca aat gac cct cgt tac gcg gac   499
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ctc gac gta gtt cca cgc acc gaa tgc ctc aag gac gtt gtg gtt cgt   547
Leu Asp Val Val Pro Arg Thr Glu Cys Leu Lys Asp Val Val Val Arg
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ttt gtt cct tac ttc gag gaa gaa atc ctg cca cgc gca aag aag ggc   595
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gaa acc gtc ctc atc gca gca cac ggc aac tcc ctg cgt gcg ctg gtt   643
Glu Thr Val Leu Ile Ala Ala His Gly Asn Ser Leu Arg Ala Leu Val
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aag cac ctt gac ggc atc tcc gat gct gat atc gca gag ctc aac atc   691
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cca acc ggc atc cca ctg gtc tac gaa atc gcc gaa gac ggt tcc gta   739
Pro Thr Gly Ile Pro Leu Val Tyr Glu Ile Ala Glu Asp Gly Ser Val
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gta aac cca ggc ggc acc tac ctc gat cct gag gca gca gca gcc ggc   787
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ctc                                                               840
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Gln Gly Glu Ala Glu Ala Lys Gly Val Leu Pro Gly Val Val Tyr Thr
        35                  40                  45

Ser Leu Leu Arg Arg Ala Ile Arg Thr Ala Asn Ile Ala Leu Asn Ala
    50                  55                  60

Ala Asp Arg His Trp Ile Pro Val Ile Arg Asp Trp Arg Leu Asn Glu
65                  70                  75                  80

Arg His Tyr Gly Ala Leu Gln Gly Leu Asp Lys Ala Ala Thr Lys Glu
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                85                  90                  95

Lys Tyr Gly Asp Asp Gln Phe Met Glu Trp Arg Arg Ser Tyr Asp Thr
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Pro Pro Pro Glu Leu Ala Asp Asp Ala Glu Tyr Ser Gln Ala Asn Asp
        115                 120                 125

Pro Arg Tyr Ala Asp Leu Asp Val Val Pro Arg Thr Glu Cys Leu Lys
    130                 135                 140

Asp Val Val Val Arg Phe Val Pro Tyr Phe Glu Glu Glu Ile Leu Pro
145                 150                 155                 160

Arg Ala Lys Lys Gly Glu Thr Val Leu Ile Ala Ala His Gly Asn Ser
                165                 170                 175

Leu Arg Ala Leu Val Lys His Leu Asp Gly Ile Ser Asp Ala Asp Ile
            180                 185                 190

Ala Glu Leu Asn Ile Pro Thr Gly Ile Pro Leu Val Tyr Glu Ile Ala
        195                 200                 205

Glu Asp Gly Ser Val Val Asn Pro Gly Gly Thr Tyr Leu Asp Pro Glu
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                                              Met Thr Gln Thr Ile
                                                1               5

gtc cat cta gtt cgc cac ggc gaa gtc cac aac cca gag aaa atc ctg   163
Val His Leu Val Arg His Gly Glu Val His Asn Pro Glu Lys Ile Leu
                10                  15                  20

tac gga cgc atg ccc gga tac agg ttg tct tcc cgt gga cgc agc caa   211
Tyr Gly Arg Met Pro Gly Tyr Arg Leu Ser Ser Arg Gly Arg Ser Gln
            25                  30                  35

gcc gcc cgc act gca gct tct ttt gaa ggc cac gat gtc acc tac att   259
Ala Ala Arg Thr Ala Ala Ser Phe Glu Gly His Asp Val Thr Tyr Ile
        40                  45                  50

gcg gcc tcc cca ttg cag cgt gtg cag gaa acc tcc gaa ccg ttc atc   307
Ala Ala Ser Pro Leu Gln Arg Val Gln Glu Thr Ser Glu Pro Phe Ile
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